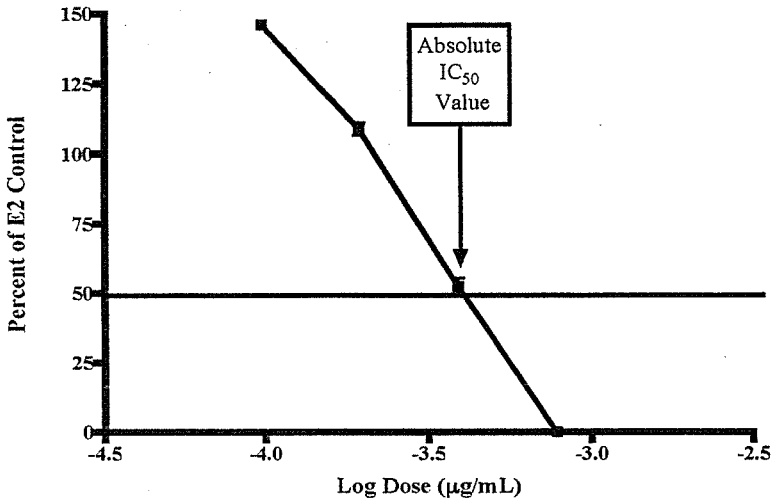
Figure 13-2 Example Concentration Curve for Calculation of Absolute IC_{50} Value

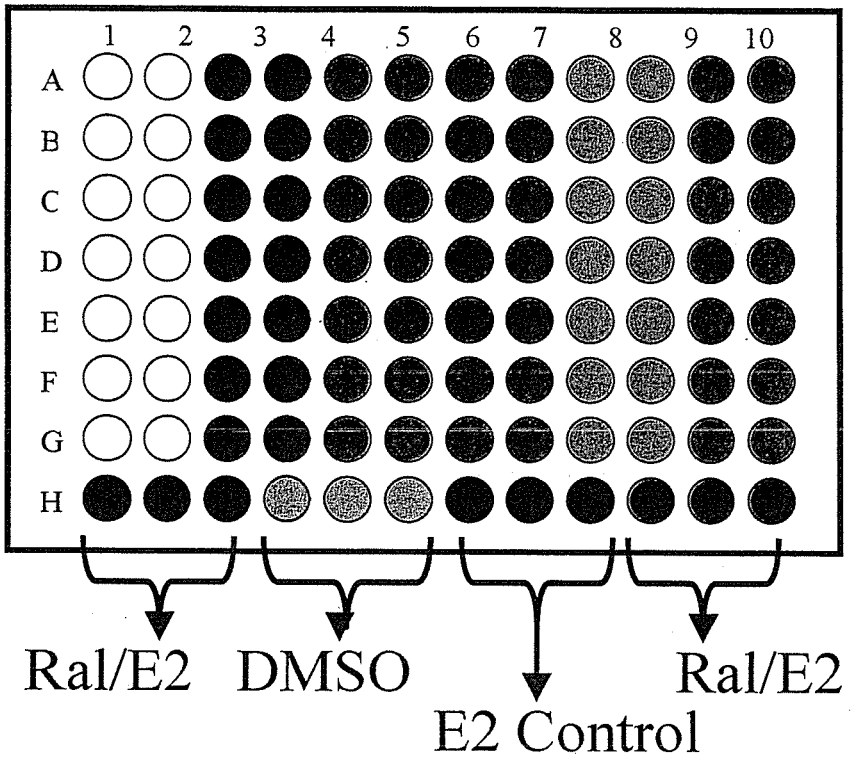


1. Convert the test substance data to the percentage of the maximum antagonist response for that substance.
2. Find the concentration of test substance that is 50% of the maximum response for that substance.

14.0 RANGE FINDER TESTING

Antagonist range finding for coded substances consists of six point, logarithmic serial dilutions tested in duplicate wells of the 96-well plate. **Figure 14-1** contains a template for the plate layout used in antagonist range finder testing.

1077 **Figure 14-1 Antagonist Range Finder Test Plate Layout**



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- **Three Point Ral/E2 Reference Standard – Replicate 1**
- **Three Point Ral/E2 Reference Standard – Replicate 2**
- **DMSO Control (1% v/v)**
- **E2 Control (2.5 x 10^{-5} µg/mL)**
- **Range Finder for Sample #1**
- **Range Finder for Sample #2**
- **Range Finder for Sample #3**
- **Range Finder for Sample #4**
- **Range Finder for Sample #5**
- **Range Finder for Sample #6**

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Evaluate whether range finder experiments have met the acceptance criteria (see **Section 13.6.3**) graph the data as described in the NICEATM Prism® users guide.

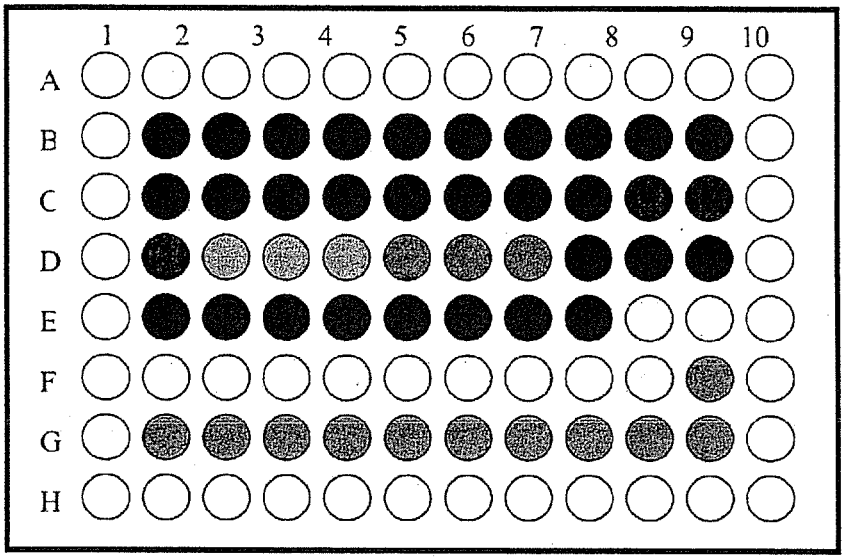
To determine starting concentrations for comprehensive testing use the following criteria:

- If there are no points on the test substance concentration curve that are less than the mean value of the E2 control minus three times the standard deviation from that mean, the highest concentration used in comprehensive testing is the limit dose or the maximum soluble dose.
- If there are points on the test substance concentration curve that are below the mean value of the E2 standard control minus three times the standard deviation from that mean, select a concentration that is a single log dilution higher than the concentration giving the lowest adjusted RLU value in the range finder and use that as the highest concentration for comprehensive testing.
- If a substance exhibits a biphasic concentration curve, the range finder experiment should be repeated unless the proposed concentration range for the comprehensive studies will include all concentrations of the biphasic region in the range finding study. If the range finder experiment is repeated and the substance still exhibits a biphasic concentration curve, comprehensive testing must be conducted on the peak of the biphasic curve at the lowest test substance concentration. If the substance is negative at this lowest concentration, then test at the higher concentration. For either peak of the concentration curve, select a concentration that is a single log dilution higher than the concentration giving the lowest adjusted RLU value in the range finder and use that as the highest concentration for comprehensive testing.

15.0 COMPREHENSIVE TESTING

Antagonist comprehensive testing for coded substances consists of 11 point, double serial dilutions, with each concentration tested in triplicate wells of the 96-well plate. **Figure 15-1** contains a template for the plate layout to be used in antagonist comprehensive testing.

Figure 15-1 Antagonist Comprehensive Test Plate Layout



- 9 point Ral/E2 Reference Standard Dose Response Curve

- Flavone Control (25 µg/mL)

- DMSO Control (1%)

- E2 Control (2.5 x 10^{-5} µg /mL)

- Comprehensive Dose Response Sample #1, Replicate #1

- Comprehensive Dose Response Sample #1, Replicate #2

- Comprehensive Dose Response Sample #1, Replicate #3

- Media only wells, not used for assay

Evaluate whether comprehensive experiments have met acceptance criteria (see **Section 13.6.3**) and graph the data as described in the NICEATM Prism® users guide.

- If the substance has been tested up to the limit dose or the maximum soluble dose without causing a significant decrease in cell viability, and there are no points on the concentration curve that are below the line indicating the mean minus three times the standard deviation of the E2 control, the substance is considered negative for antagonism.

- If the substance has been tested up to the limit dose and there are points on the concentration curve that are below the line indicating the mean minus three times the standard deviation of the E2 control, but cell viability, as measured by CellTiter-Glo® falls below 80%, or a visual inspection score of 2 or greater, at all points falling below the E2 line, the substance is considered negative for antagonism.
- If there are points on the test substance concentration curve that are below the line indicating the mean minus three times the standard deviation of the E2 control that do not cause a decrease in cell viability below 80% as measured by CellTiter-Glo®, or a visual inspection score of 2 or greater, the substance is positive for antagonism.
    - Points in the test substance concentration curve that cause a decrease in cell viability to below 80% as measured by CellTiter-Glo® or a visual inspection score of 2 or greater, are not included in data analyses.
    - Calculate an absolute IC_{50} value for all positive substances.
    - If the concentration curve for a positive substance reaches saturation at the highest and lowest concentrations tested, calculate a relative IC_{50} value.

16.0 USE OF THE HISTORICAL DATABASE TO GENERATE QC CHARTS

The historical database is maintained in order to ensure that the test method is functioning properly. The historical database is maintained as an Excel® spreadsheet that is separate from the spreadsheets used to report the data for individual experiments. The controls used to develop the historical database are used as one of the criteria for determining a valid test.

Results collected during Phase I will be compared to historical control data established during the LUMI-CELL® ER Protocol Standardization Study. Reference standard and control data collected during Phase I will be used to compile the initial historical database. Reference standard and control data collected during Phase IIa will be added to the historical database compiled in Phase I and this combined historical database will be used to establish acceptance criteria for Phase II. Reference standard and control data collected during Phase IIb will be added

to the historical database compiled in Phases I and IIa and this combined historical database will be used to establish acceptance criteria for Phases III and IV.

16.1 LUMI-CELL® ER Antagonist QC Charts

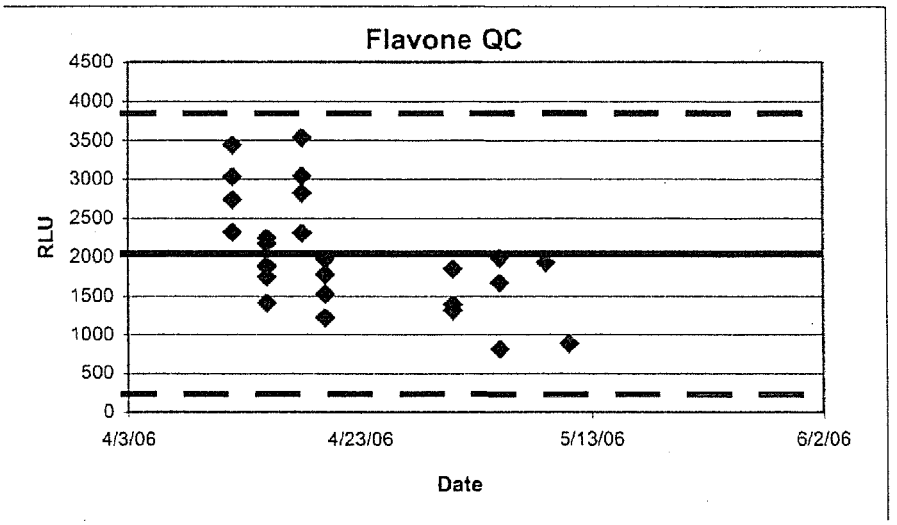
1. Open the Excel® spreadsheet labeled LUMIAntagonistQC.
2. Save this sheet under a new name, adding the laboratory designator to the file name (e.g., for Laboratory A, the new name would be ALUMIAntagonistQC).

16.1.1 Flavone/E2

1. Open Excel® spreadsheet XDSLUMIAntQC.
2. Click on flavone tab and enter the date, plate number (name), and the average 1.56×10^{-3} µg/mL RLU value for ral/E2 (data located in column F on the "List" tab of the antagonist report file).
3. Enter the three values for flavone/E2 into column D.
4. The mean and 2.5 times the standard deviation plus (and minus) the mean are calculated automatically.
5. Check the Scatter Charts tab to see if the average value for flavone/E2 falls within 2.5 times the standard deviation (e.g., **Figure 16-1**) of the historical mean. If the mean flavone/E2 value falls within the 2.5 times the standard deviation area, it passes QC. Otherwise it fails QC and the experiment must be repeated.

Acceptance or rejection of the flavone/E2 control data is based on whether the data for a given experiment falls within 2.5 times the standard deviation from the historical mean RLU value.

Figure 16-1 Example Scatter Chart of the Flavone/E2 Control QC[1,2,3]



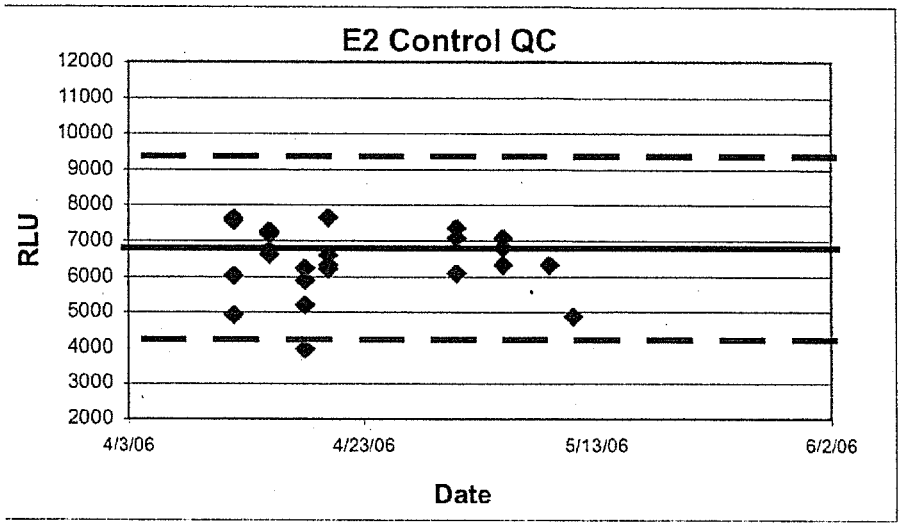
[1]Each point represents a single experiment.
[2]The solid line represents the historical mean RLU value for the flavone control.
[3]The two dashed lines represent the historical mean RLU value for the flavone control plus and minus 2.5 times the standard deviation from the historical mean.

16.1.2 E2 Control

1. Click on the E2 control tab. The date, study name, and 1.56 x 10^{-3} µg/mL ral/E2 data should be populated in the appropriate position.
2. Enter the values for the E2 control into column D.
3. The mean and 2.5 times the standard deviation plus (and minus) the mean are calculated automatically.
4. Check the scatter charts tab to see if the average value for the E2 control falls within the 2.5 times the standard deviation (**Figure 16-2**) from the historical mean. If the value falls within the 2.5 times the standard deviation area, the E2 control passes QC. Otherwise it fails QC and the study must be repeated.

Acceptance or rejection of the E2 control data is based on whether the data for a given experiment falls within 2.5 times the standard deviation from the historical mean RLU value.

Figure 16-2 Example Scatter Chart of the E2 Control QC

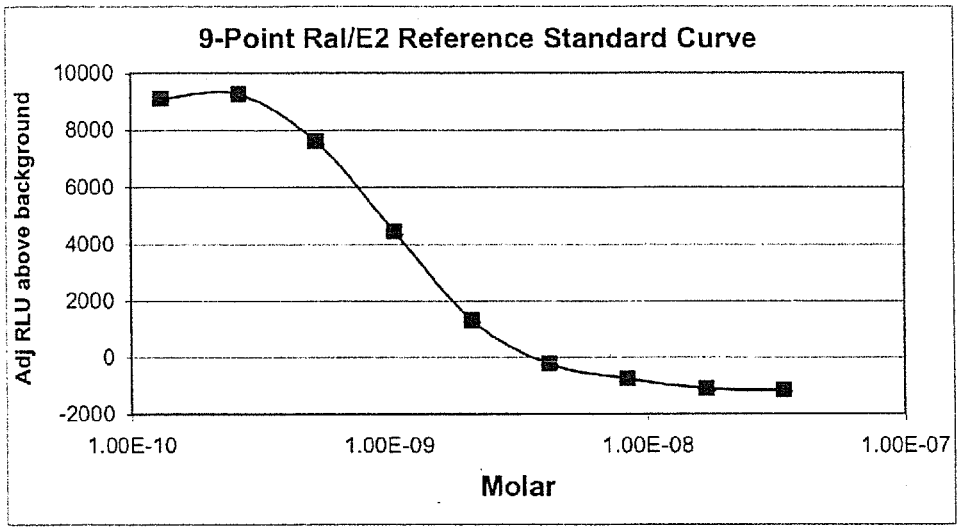


[1]Each point represents a single experiment.
[2]The solid line represents the historical mean RLU value for the E2 control.
[3]The two dashed lines represent the historical mean RLU value for the E2 control plus and minus 2.5 times the standard deviation from the historical mean.

16.1.3 9-Point Ral/E2 Reference Standard QC

1. Enter the experiment date and name, and copy and paste the adjusted RLU values for Ral/E2 into the appropriate slots on the tab labeled Ral E2 Standard Curve.
2. The Ral/E2 standard curve is automatically graphed to ensure a normal sigmoidal shape (see **Figure 16-3** for an example curve).

Figure 16-3 Example Figure of a Sigmoidal Ral/E2 Concentration Response Curve[1]

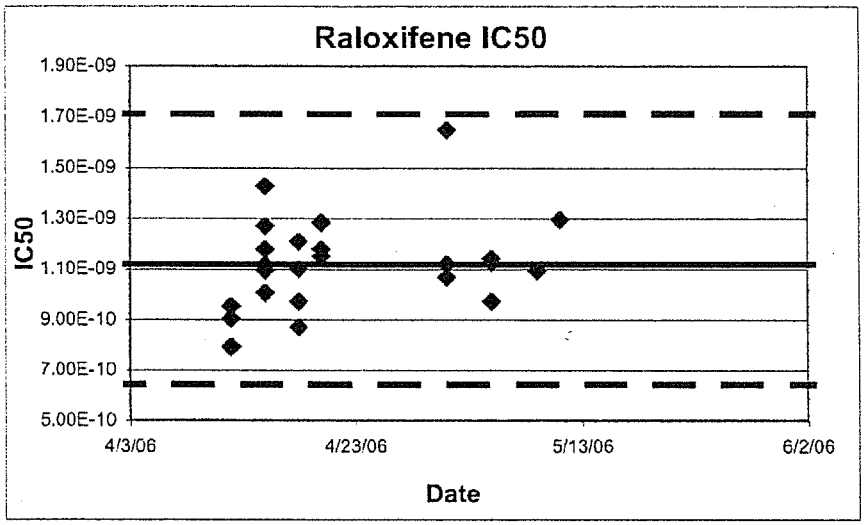


[1]The points on the line represent the averaged Ral/E2 values for a single experiment.

16.1.4 IC_{50} Tracking Data

1. Enter the date and plate ID into the first two columns of the IC_{50} Tracking Data tab.
2. Link the EC_{50} data from the 9-point Ral/E2 Curve QC tab to the column to the right of the plate information.
3. Column E calculates the percent deviation from the historical database IC_{50} value.
4. The mean and 2.5 times the standard deviation plus (and minus) the mean for the IC_{50} deviation are calculated automatically.
5. Check the Scatter Charts tab to see whether the experimental IC_{50} value falls within the 2.5 times the standard deviation (**Figure 16-4**). If the value falls within the 2.5 times the standard deviation area, it passes QC. If the value does not fall within the 2.5 times the standard deviation, it fails QC and the experiment must be repeated.

Figure 16-4 Example Scatter Chart of the Ral/E2 IC_{50} Control QC



[1]Each point represents a single experiment.
[2]The solid line represents the historical mean RLU value for the Ral/E2 EC_{50}.
[3]The two dashed lines represent the historical mean RLU value for the Ral/E2 control plus and minus 2.5 times the standard deviation from the historical mean.

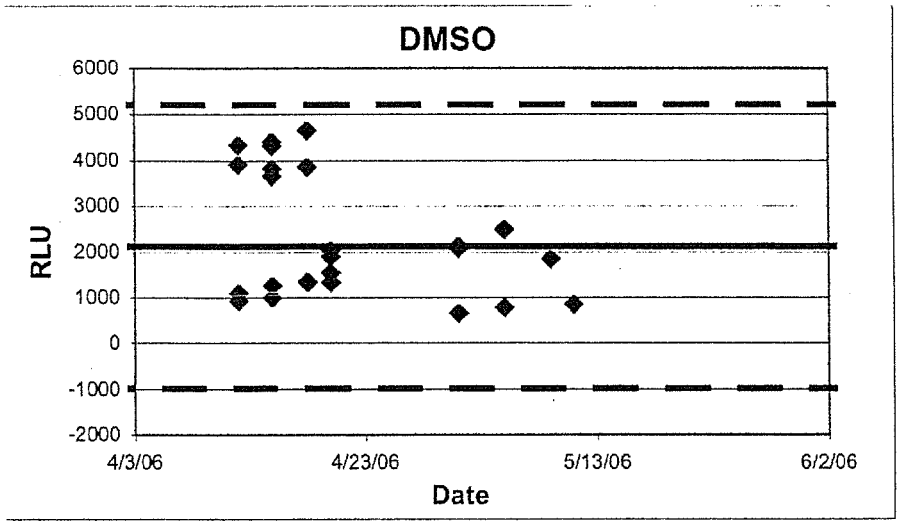
Acceptance or rejection of Ral/E2 IC_{50} data is based on whether the data falls within 2.5 times the standard deviation from the mean for the historical Ral/E2 IC_{50} RLU value.

16.1.5 DMSO

1. The date and experiment name should populate automatically
2. Enter all of the DMSO values from Table 1 on the "Raw Data" tab on the Excel® spreadsheet which passed the outlier test, into the areas marked DMSO 1, DMSO 2, DMSO 3, and DMSO 4.
3. The average RLU value for DMSO is then calculated under the "mean" column.
4. The mean and 2.5 times the standard deviation plus (and minus) the mean for the DMSO deviation are calculated automatically.
5. Check the Scatter Charts tab to see whether the average value for DMSO falls within 2.5 times the standard deviation (**Figure 16-5**) from the mean. If the value falls within the 2.5 times the standard deviation area, the DMSO passes QC. If

the value does not fall within the 2.5 times the standard deviation, it fails QC and the experiment must be repeated..

Figure 16-5 Example Scatter Chart of the DMSO Control QC[1,2,3]



[1]Each point represents a single experiment.
[2]The solid line represents the historical mean RLU value for the DMSO control.
[3]The two dashed lines represent the historical mean RLU value for the DMSO control plus and minus 2.5 times the standard deviation from the historical mean.

Acceptance or rejection of the DMSO control data is based on whether the data for a given experiment falls within 2.5 times the standard deviation from the historical mean RLU value.

16.1.6 Reduction

Reduction is calculated by dividing the highest RLU value for each of the Ral/E2 standards by the lowest Ral/E2 RLU value. To calculate reduction, follow the steps below:

Enter the reduction value from the "Raw Data" tab on the Excel® spreadsheet. If the value is greater than or equal to 3, the experiment passed QC. An induction value of less than 3 fails induction QC and the experiment must be repeated.

17.0 QUALITY TESTING OF MATERIALS

All information pertaining to the preparation and testing of media, media supplements, and other materials should be recorded in the Study Notebook.

17.1 Tissue Culture Media

Each bottle of tissue culture medium must be tested in a single growth flask of cells before use in ongoing tissue culture or experimentation.

1. Every new lot of media (RPMI and DMEM) and media components (FBS, Charcoal/Dextran treated FBS, and L-glutamine) must first be tested on the LUMI-CELL® ER assay prior to being used in any GLP acceptable assays.
2. Add 4 µL of DMSO (previously tested) into four separate 13 mm tubes.
3. Add 400 µL media (to be tested) to 13 mm tube.
4. Dose an experimental plate as in **Section 12.0,** treating the media being tested as a test substance.
5. Analyze 96-well plate as described in **Section 12.0,** comparing the data from the DMSO controls made using previously tested tissue culture media to the new media being tested.
6. Use the QC charts to determine if the new media with DMSO lies within 2.5 standard deviation of the mean for the media. If the RLU values for the new media with DMSO are within 2.5 standard deviation of the mean for the historical data on DMSO, the new lot of media is acceptable. If the RLU values for the new media with DMSO are not within 2.5 standard deviation of the mean for the historical data the new lot may not be used in the assay.
7. Note date and lot number in study notebook.
8. If the new bottle passes the QC as described in **Section 15.1 step 6**, apply the media to a single flask cells and observe the cells growth and morphology over the following 2 to 3 days. If there is no change in growth or morphology, the new media is acceptable for use.

17.2 G418

1. New lots of G418 must first be tested on the LUMI-CELL® ER assay prior to being used in any GLP acceptable assays.

2. Add 220 µL of G418 (previously tested) to a single flask containing cells growing in RPMI.
3. Add 220 µL of G418 (to be tested) to a different flask containing cells growing in RPMI.
4. Observe cellular growth and morphology in both tissue culture flasks over a 48 to 72 hour period. If there are no differences in observed growth rate and morphology between the two flasks, the new G418 lot is acceptable.
5. If cellular growth is decreased, or the cells exhibit abnormal morphology, the new lot of G418 is not acceptable.
6. Note date and lot number in study book.

17.3 DMSO

1. Every new bottle of DMSO must be tested on the LUMI-CELL® ER assay prior to use in any GLP acceptable assays.
2. Add 4 µL of DMSO (to be tested) into four separate 13 mm tubes.
3. Add 400 µL media (previously tested) the same tubes.
4. Dose an experimental plate as in **Section 15.0,** treating the media being tested as a test substance.
5. Analyze 96-well plate as described in **Section 15.0,** comparing the data from the DMSO controls made using previously tested tissue culture media to the new media being tested.
6. Use the QC charts to determine if the new media with DMSO lies within 2.5 standard deviation of the mean for the media. If the RLU values for the new media with DMSO are within 2.5 standard deviation of the mean for the historical data on DMSO, the new lot of media is acceptable. If the RLU values for the new media with DMSO are not within 2.5 standard deviation of the mean for the historical data the new lot may not be used in the assay.

7. Use the QC charts to determine if the new DMSO is within 2.5 standard deviation of the mean for DMSO background. If the RLU for the new DMSO is within 2.5 standard deviation of the mean for the historical data on DMSO, then the new bottle of DMSO is acceptable; otherwise the new bottle may not be used in the assay.

8. Note the date, lot number, and bottle number in study book.

9. If no DMSO has been previously tested, test several bottles as described in **Section 15.3**, and determine whether any of the bottles of DMSO have a higher average RLU than the other bottle(s) tested. Use the DMSO with the lowest average RLU for official experiments.

17.4 Plastic Tissue Culture Materials

1. Grow one set of cells, plate them for experiments on plastic ware from the new lot and one set of cells in the plastic ware from a previous lot, and dose them with E2 reference standard and controls.

2. Perform the LUMI-CELL® ER experiment with both sets of cells.

3. If all of the analysis falls within acceptable QC criteria, then the new manufacturer's products may be used.

18.0 REFERENCES

Eli Lilly and Company and National Institutes of Health Chemical Genomics Center. 2005. Assay Guidance Manual Version 4.1. Bethesda, MD: National Institutes of Health. Available: http://www.ncgc.nih.gov/guidance/manual_toc.html [accessed 05 September 2006]

ICCVAM. 2001. Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity. NIH Pub. No. 01-4500. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/invidocs/guidance/iv_guide.pdf [accessed 31 August 2006]

DRAFT REPORT OF THE PRELIMINARY VALIDATION ASSESSMENT PANEL OF THE 'JAPANESE MULTI-LABORATORIES VALIDATION STUDY OF A STABLY TRANSFECTED ER ALPHA MEDIATED REPORTER GENE ASSAY IN JAPAN'.

Final version 29 June 2006

ACCRONYMS

AR Androgen Receptor

CERI Chemicals Evaluation and Research Institute (Japan)

CV coefficient of variation

DIP Data interpretation procedure

ECVAM European Centre for the Validation of Alternative Methods

EDTA (OECD) Task Force on Endocrine Disruptor Testing and Assessment

ER Estrogen Receptor

ERE Estrogen Responsive Element

GD 34 Guidance Document 34

GLP Good Laboratory Practice

ICCVAM Interagency coordinating Committee on the Validation of Alternative Methods (US)

JaCVAM Japanese Centre for the Validation of Alternative Methods

NICEATM National Toxicology Program (NTP) Interagency Centre for the Evaluation of Alternative Toxicological Methods (US)

NIEHS National Institute of Environment and Health Sciences (US)

NIHS National Institute of Health Sciences (Japan)

PC_{50}/PC_{10} The concentration of chemical estimated to cause 50% or 10%, respectively, of activity of the positive control response on a plate by plate basis.

PM Prediction Model

QA Quality Assurance

SOP Standard Operating Procedure

SPSF Standard Project Submission Form

TA Transcriptional Activation

US EPA US Environmental Protection Agency

VMG-NA Validation Management Group for Non –Animal Testing

WNT (OECD) Working Group of the National Coordinators for the Test Guidelines Programme

1. INTRODUCTION AND BACKGROUND

1.1 At the present time, there is global concern regarding endocrine disruption effects, particulary mediated by the estrogen receptor (ER) resulting from chemical exposure. Several *in vitro* ER binding and transfected cell line assay methods are currently or imminently being (pre) validated at national, regional and international levels, but are some way away from completion and full assessment of their validation status.

1.2 A screening test method is a rapid, usually simple test performed for the purposes of prioritizing or grouping substances in general categories of potential modes of action (*e.g., in vitro* binding to the oestrogen receptor). The results from screening tests are generally used for preliminary decision making and to set priorities for additional and more complex tests. Although the results from screening tests, alone, may not be sufficient for risk assessment purposes, there may be circumstances where such results may be combined with other test results in a tiered testing approach to provide in the hazard/risk assessments (GD34).

1.3 Currently, no *in vitro* screening assay for ER activity that can be used for OECD regulatory purposes has been peer reviewed for potential test guideline development, although the need is urgent. Recognizing this urgency, Japan has made an extensive effort to establish and domestically validate a new *in vitro* pre-screening procedure, the **hER-HeLa-9903 Estrogen Receptor (ER) Transcriptional Activation (TA) Test** for detecting the estrogenic activity of chemicals for a level 2 screening test in the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals.

1.4 The with-in Japan multi-laboratory validation process of Japanese ER TA Assay was completed as an activity of the Validation Management Group (Non -Animal)(VMG-NA) and the results were presented at the 3rd VMG-NA held in November 2005.

1.5 The assay is based on an estrogen reactive stable human cervical tumor cell line, hER-HeLa- 9903, which was developed by the Sumitomo Chemical Company in Japan. An initial test protocol of the assay system was developed and optimized by the Chemicals Evaluation and Research Institute (CERI). Using this optimized protocol, a pre-validation of the test system was conducted by CERI as an initial assessment exercise in order to identify the reliability, relevance and performance (accuracy) of the assay system. Following this first assessment, CERI, led an inter-laboratory validation involving four participating laboratories, all of which used coded chemicals under GLP compliance conditions. The data produced indicated good reproducibility and technical transference between laboratories. The data compared favourably and showed good concordance with that reported for the immature rat uterotrophic assay (80%) and summarised by ICCVAM (85%), (ICCVAM 2003), with an overall low false positive rate of 9%.

1.6 Following this presentation, the VMG-NA agreed to create a panel with the task of assisting the Japanese in assessing the readiness of the validation study for independent scientific peer review and

supporting additional requirements that might be deemed necessary. The panel activities were informal and unofficial, as member countries did not make official nominations for panel membership, and the panel members participated on a voluntary basis.

1.7 Using GD 34 criteria as a basis, the primary tasks or charges of the panel were to assist the Japanese in a transparent manner in assessing whether there is sufficient information on the domestic validation to submit a report for scientific review, with the independent review procedure to be agreed by the Japanese. This report, which is based upon the three teleconference discussions of the panel held over six months, provides the first step in this process and will be made available to the VMG-NA. **All the points and discussions documented herein were agreed by the panel during their preliminary validation assessment activities or charge.** These teleconferences were conducted under the auspices of the individual expertise of the participants, and therefore the teleconference minutes and this report reflect their expert opinion, and not that of the organisations in which the experts are employed.

1.8 The report outlines the panel discussions, and each meeting is summarised in this report. Through the teleconference process the steps taken in the preliminary validation assessment are identified, and also included as appendices are the summary statements from participants. Subsequent activities include writing of a comprehensive validation report, or peer charge, using the preliminary validation assessment report as a basis. Following this next report the Japanese will decide how to go ahead with the formal peer review process. Routes for the organisation of independent peer review were identified and discussed at the 9th EDTA and 18th WNT via a contract house or by a member country competent authority, as follows: 'The Secretariat drew the attention of the WNT on Document ENV/JM/TG(2006)5 including two examples of approaches proposed to address the peer review of validated methods: a proposal made by the United States and a proposal made by Japan. It proposed to initially address peer reviews on a case-by-case basis until experience is gained and after a certain time, possibly consider a more comprehensive guidance on the processes for peer review. The United States introduced Annex 1 of Document ENV/JM/TG(2006)5 , which does not apply to a specific assay. The Secretariat brought Information Document [INF.6] to the attention of the meeting, as a collation of comments received from members of the VMG-eco on the Annex 1 of Document ENV/JM/TG(2006)5. The WNT agreed that the document describes a plausible approach, but does not provide standard procedures.' (Paragraph 36 of the Draft Summary record of the 18th meeting of the WNT, Bern Switzerland 16-18 May 2006.)

1.9 Should the assay be ultimately considered by the Japanese to be appropriate for submission to the OECD, the Japanese will be required to submit a SPSF to the OECD Secretariat for consideration by the WNT. On 1 June 2006, in response to queries from CERI, and discussions and recommendations at the 18th WNT with the Japanese National Coordinator and chair, the Secretariat recommended that the Japanese submit an SPSF soon, so that the project could be added to the rolling work plan.

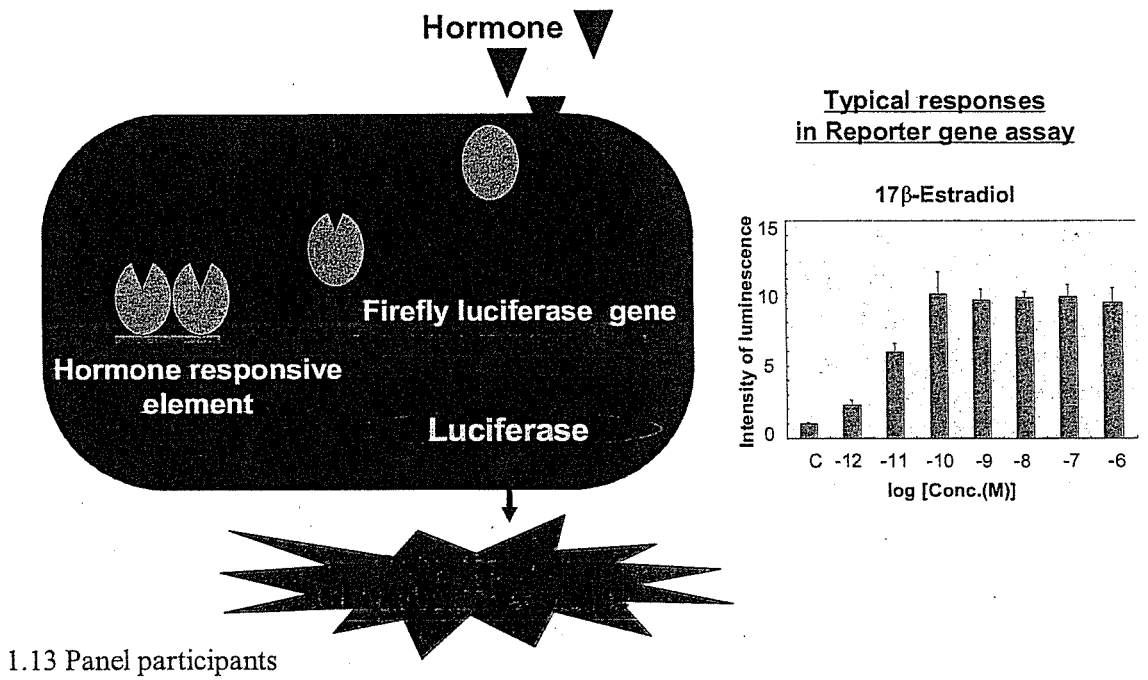
Background information: What is a reporter gene assay?

1.10 The Reporter gene assay method is an *in vitro* tool that allows the identification of promoters and enhancers together with an assessment of the correlations between their activities and conformations by measurement of the reporter proteins that are expressed from reporter genes. The promoters and the enhancers, which are upstream of all protein coding regions on the genome, adjust the activity and enhancement of the expression of the proteins. Because the reporter genes that code useful proteins that become indicators later in the target cells are artificially built downstream of the promoters and enhancers, reporter genes have become a focus of investigations. In the case of luciferase (a gene from the firefly), if a substrate is added to the cells expressing this enzyme, bioluminescence is observed so the expression from the reporter gene is detected visually and can also be measured quantitatively (See Figure 1).

1.11 Thus the reporter gene assay technique may be suitable for detecting hormonal activity of chemicals, because it has been used to detect enhancers and promoter activity of genes. The reporter gene assay system may also provide a powerful tool to screen for endocrine disrupting chemicals (Takeyoshi et al., 2002; Yamasaki et al., 2002, and has also been developed for use in other cell lines, e.g. CALUX (Sonneveld et al 2006).

1.12 The assay used for this validation study uses the human cervical tumor cells host cell line HeLa cell line with an inserted construct: Human ERα expression vector (full-length) with a firefly luciferase reporter construct bearing five tandem repeats of a vitellogenin estrogen-responsive element (ERE) driven by a mouse metallothionein promoter TATA element.

Figure 1. Diagram showing the principle of the reporter gene assay



1.13 Panel participants

The panel participants were proposed during and following the 3rd VMG-NA, on the basis of the organisation they represented at the VMG-NA, and for their specific expertise particularly in relation to statistical analyses, validation and /or receptor screening assays. The panel initially included the following experts:

1. Dr. Masahiro Takeyoshi (CERI)
2. Dr. Yumi Akahori (CERI)
3. Prof. Daniel Dietrich (on behalf of ECVAM)
4. Dr. Susan Laws (US EPA)
5. Mr. Gary Timm (US EPA)

6. Dr. Yutaka Aoki (ASPH Fellow at US EPA)

7. Dr.Tim Schrader (Health Canada)

8. Dr. Bill Stokes (NIEHS/NICEATM, ICCVAM)

9. Dr. Ray Tice (NIEHS/NICEATM, ICCVAM)

10. Ms. Patricia Ceger (ILS. Inc./NICEATM, ICCVAM)

11. Mr. Frank Deal (ILS. Inc./NICEATM, ICCVAM)

12. Dr. Miriam Jacobs (OECD call leader)

There were alterations in participation of the panel activities: Following the first teleconference,

13. Dr. Jun Kanno (NIHS) and

14. Dr. Hajime Kojima (JaCVAM)

were invited to join, to improve the Japanese representation and expertise.

Although a panel member, Prof Bob Combes did not participate in any of the teleconferences, but did submit written comments at a later date.

1.14 Following the second teleconference it became apparent that the panel had become a little unbalanced with respect to numbers of persons with validation expertise representing different bodies. The Secretariat therefore recommended that the numbers of such persons for each of the different participating bodies, during the teleconferences, is reduced and/or maintained at two persons to improve the balance in representation across the participating bodies and improve manageability of the teleconference.

1.15 Further consultation with experts outside the panel was sought where panel members felt it useful. Dr Ray Tice requested further consultation on statistical matters from the Statistical expert consultant to ICCVAM, Dr. Joe Haseman. The ECVAM computational toxicology and statistical expert Dr. Sebastian Hoffman was also consulted. Dr. Jean-Claude Nicholas was consulted with respect to the possibility of induction of non receptor mediated effects that might be observed at higher concentrations that might impact upon and increase the chemical luminescence.

Steps undertaken during preliminary validation assessment process

1.16 The steps taken during the preliminary validation assessment at the request of the panel included (in chronological order):

- Teleconference 2

  i. CERI conducted a comparison of the draft report submission with the guidelines provided in the OECD Guidance Document 34 and *ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors* (NIH Pub. No. 03-4503) and stated their rationale for deviations from these guidelines.

  ii. CERI provided further information on cell line characterisation, methods of cytotoxicity evaluation and ER alpha antagonist TA testing (See also appendix 1).