NICEATM

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

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods

Tox21 Adaptation of the BG1 ER TA Test Method: Preliminary Assessment of Accuracy

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NIEHS/NTP

SACATM Meeting

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National Institute of Environmental Health Sciences

Research Triangle Park, NC

National Toxicology Program

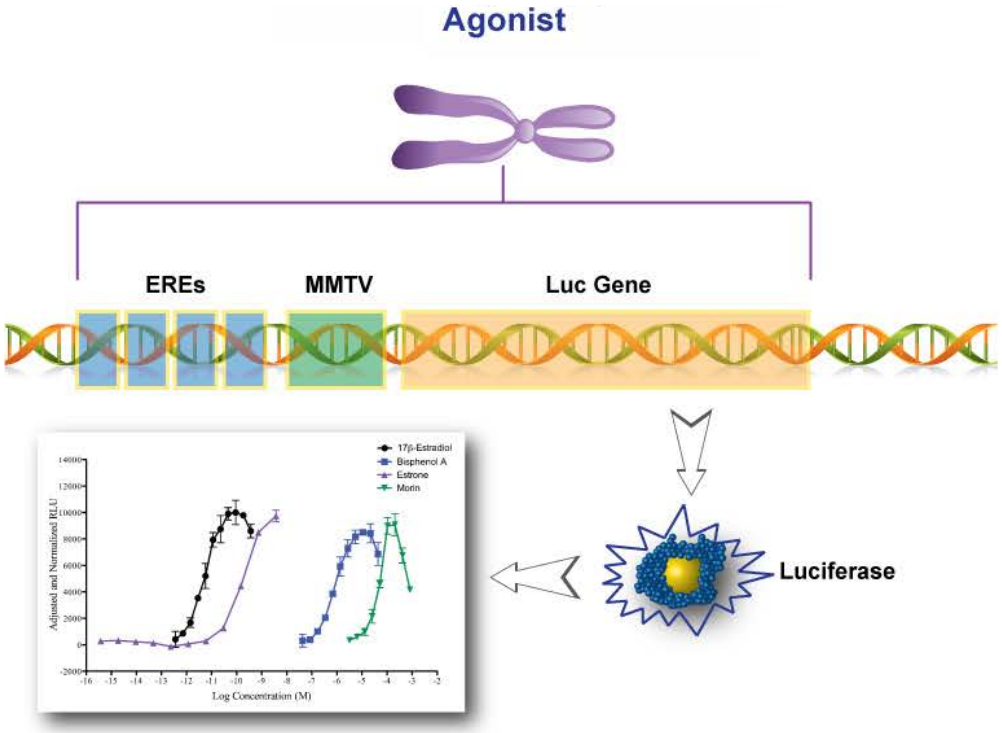
U.S. Department of Health and Human Services

ICCVAM Recommendations, BG1 TMER:

- "ICCVAM encourages users to provide all data that are generated from future studies to ICCVAM so that they may be used to further characterize the usefulness and limitations of the BG1Luc ER TA test method as a screening test to identify substances with *in vitro* ER agonist or antagonist activity."

BG1Luc ER TA (LUMI-CELL®) Assay

- Human ovarian carcinoma cell line (BG1) with reporter gene construct
- Estrogen receptor (ER) agonist and antagonist protocols
- NIEHS SBIR-funded development (XDS, Inc)
- NICEATM (lead), ECVAM, and JaCVAM sponsored validation labs; started 2007, completed 2010



Toxicology Testing in the 21st Century

- Toxicology Testing in the 21st Century (Tox21) is a collaboration between:
  - National Institute of Environmental Health Sciences/National Toxicology Program
  - National Center for Advancing Translational Sciences (NCATS)
  - Environmental Protection Agency
  - Food and Drug Administration

Tox21 Goals

- Research, develop, validate, and translate test methods that characterize toxicity pathways
- Identify compounds, assays, informatic tools, and targeted testing needed for the test methods
- Prioritize compounds for more extensive toxicological evaluation
- Identify mechanisms of toxicity to characterize toxicity pathways, facilitate cross-species evaluation, and provide input to models for low-dose extrapolation
- Develop predictive models for biological response in humans
- Tox21 plans to test 10,000 chemicals in ~50 assays per year

Current Tox21 Test Methods

- Apoptosis
- Cell viability
- DNA damage
- Mitochondrial toxicity
- Nuclear receptors
  - Including human androgen and estrogen receptor methods
- Other toxicity Pathways

Development of the BG1Luc ER TA qHTS Assay

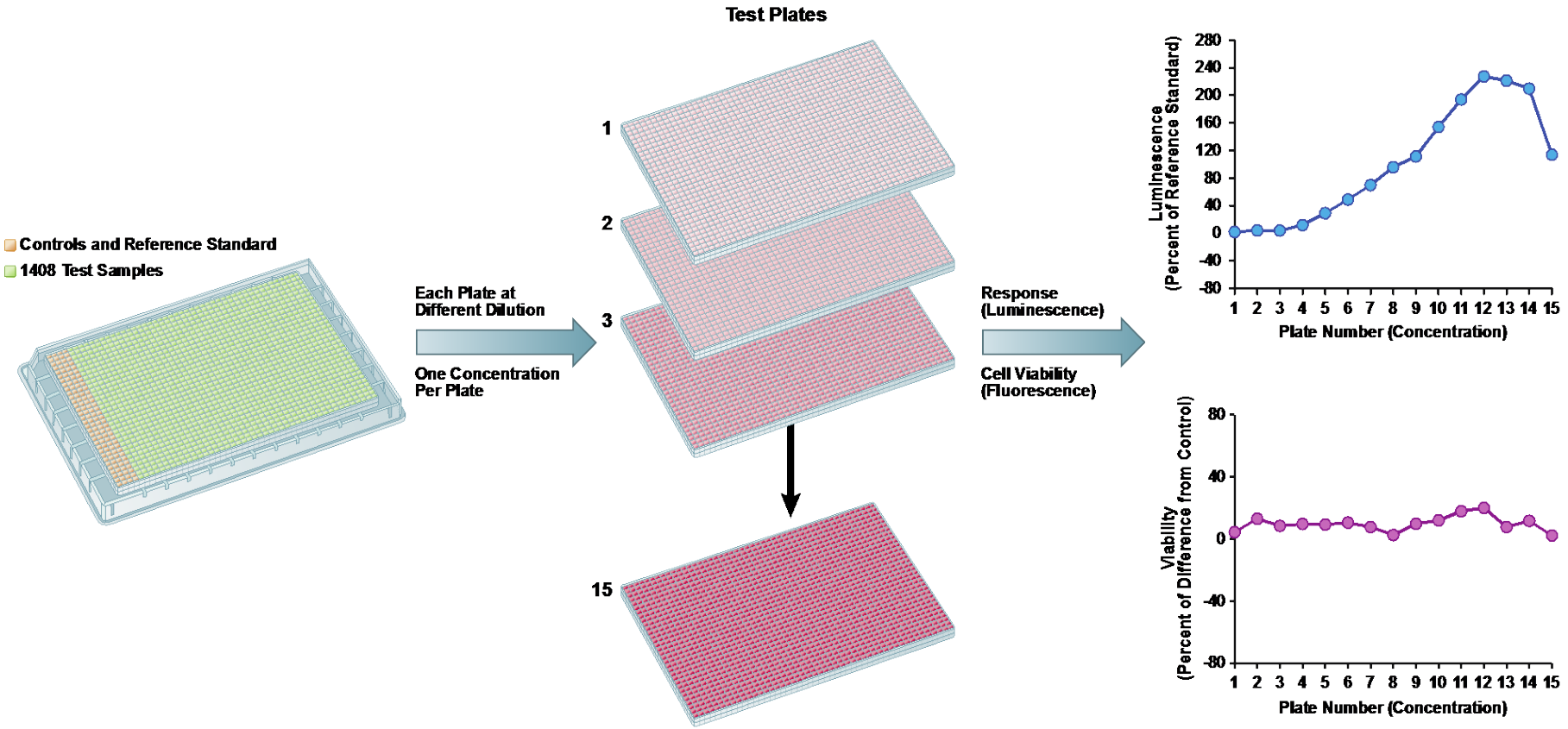
- Adapted for Tox21 Screening at NCATS

96-well (hand pipette) ⟶ 1536-well (fully automated)

- First assay validated for regulatory use to be adapted to qHTS by Tox21*

*This does not mean that the qHTS method is validated for regulatory use.

BG1Luc Experimental Design: Quantitative High Throughput Screening (qHTS)



Comparison of BG1 Manual and qHTS Procedures

BG1 Manual	BG1 qHTS
96-well plate	1536-well plate
2 test substances per plate	1408 test substances per plate
11 point, serial dilution (1 plate)	15 point, serial dilution (15 plates)
Concentrations determined by range finder, followed by focused testing (~3 log range) up limit of solubility or 1 mM	Fixed concentrations typically ranging from 0.5 nM to 92 µM
Each test substance tested in triplicate in each experiment	Each test substance tested once in each experiment (each experiment run x3)
40,000 cells per well	4,000 cells per well
200 µL per well	10 µL per well
Wash steps	No wash steps
7-14 Days	3 Days
Viability determined visually	Viability determined by fluorescent marker

BG1Luc ER TA qHTS data

- 11,776 substances (8188 unique) tested 3x in BG1Luc ER TA qHTS agonist and antagonist assays

Tox21 10K Library

NCGC	EPA	NTP
• Drugs	•ToxCast I and II compounds	•NTP-studied compounds of all types
• Drug-like compounds	•Antimicrobial Registration Program	•NTP nominations and related compounds
• Active pharmaceutical ingredients	•Endocrine Disruptor Screening Program	•ICCVAM and NICEATM validation and reference compounds
	•OECD Molecular Screening Working Group List	•Outside collaborators (e.g., U.S. Army Public Health Command)
	•Failed Drugs	

BG1Luc ER TA qHTS data

- 11,776 substances (8188 unique) tested 3x in BG1Luc ER TA qHTS agonist and antagonist assays
  - Included most substances from NICEATM validation study
  - Data from both methods were used to evaluate accuracy and concordance of the qHTS method relative to the validated BG1 manual method.

NICEATM Review of qHTS Data

- Applied criteria developed for the BG1 Manual Assay to the BG1 qHTS data
  - qHTS concentration-response curves reviewed using a defined set of parameters for assessing activity (Positive, Negative, AC_{50})

[1]Based on method used in ICCVAM. 2011. ICCVAM Test Method Evaluation Report on the LUMI-CELL® ER (BG1Luc ER TA) Test Method An In Vitro Method for Identifying ER Agonists and Antagonists. NIH Publication No. 11-7850. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

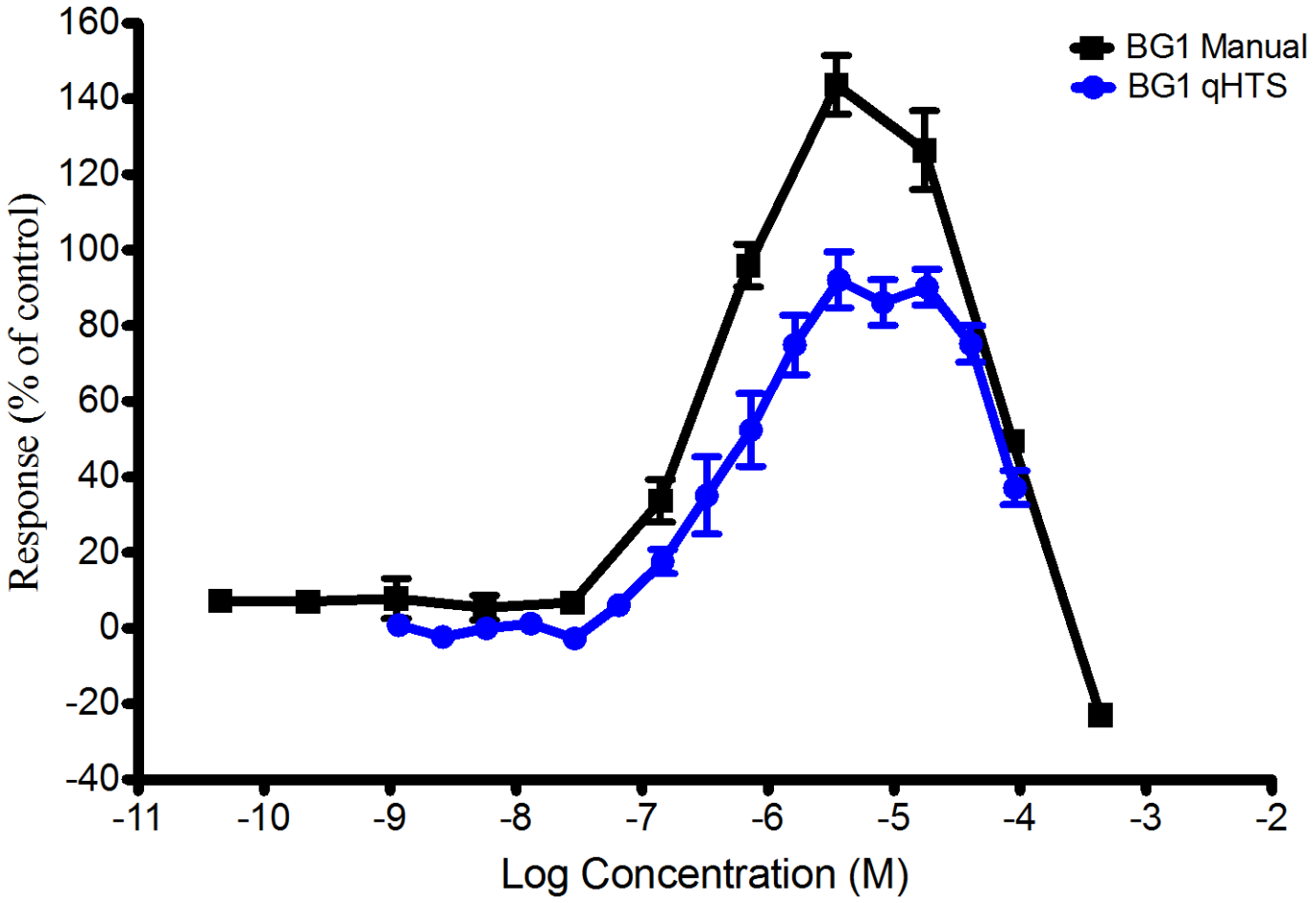
Agonist Concordance

- Agonist concordance was calculated for 61 substances

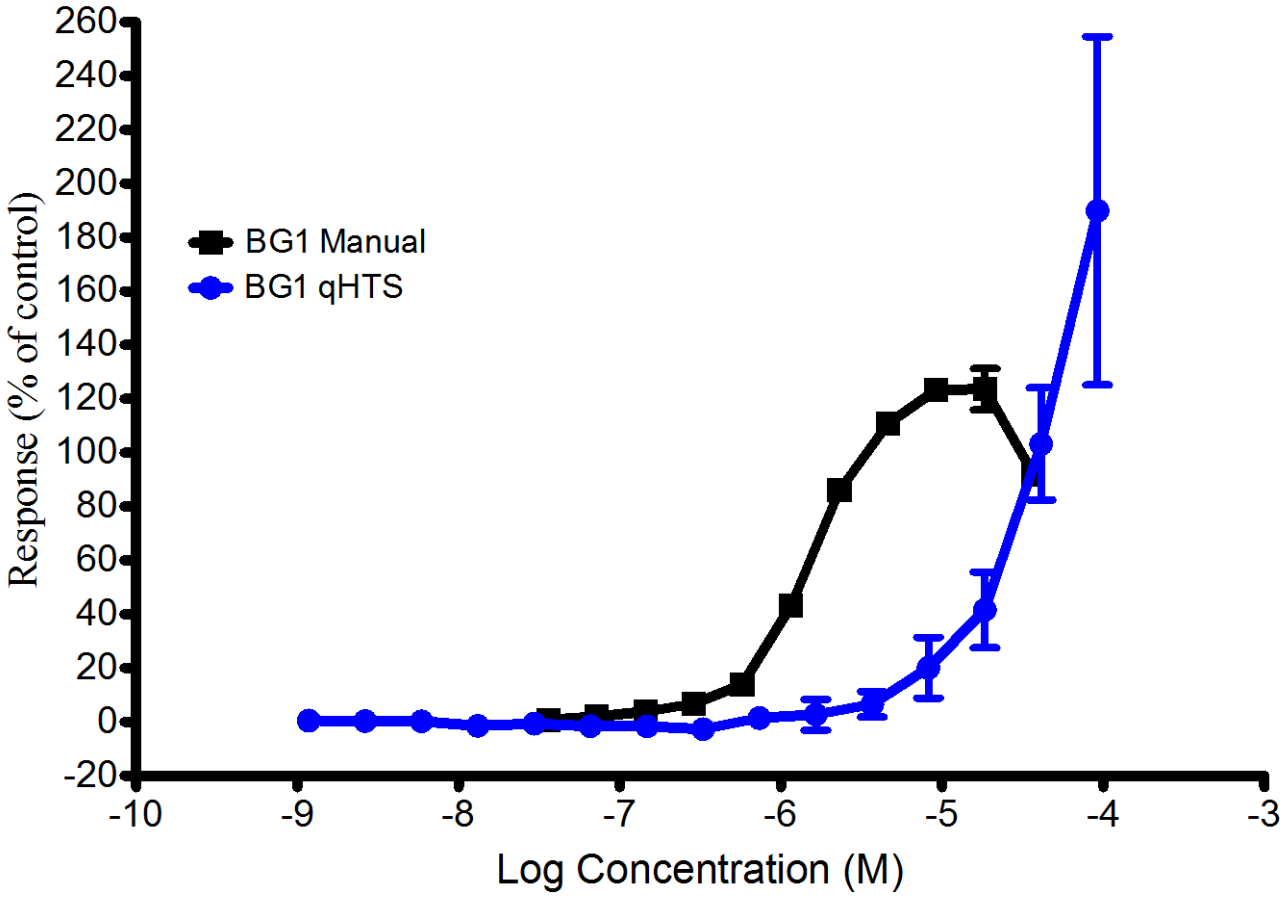
		BG1 qHTS Classification	
		Positive	**Negative**
BG1 Validation Classification	**Positive**	**31**	**4**
	Negative	**0**	**26**

- Concordance between the manual and qHTS assays was 93% (57/61)

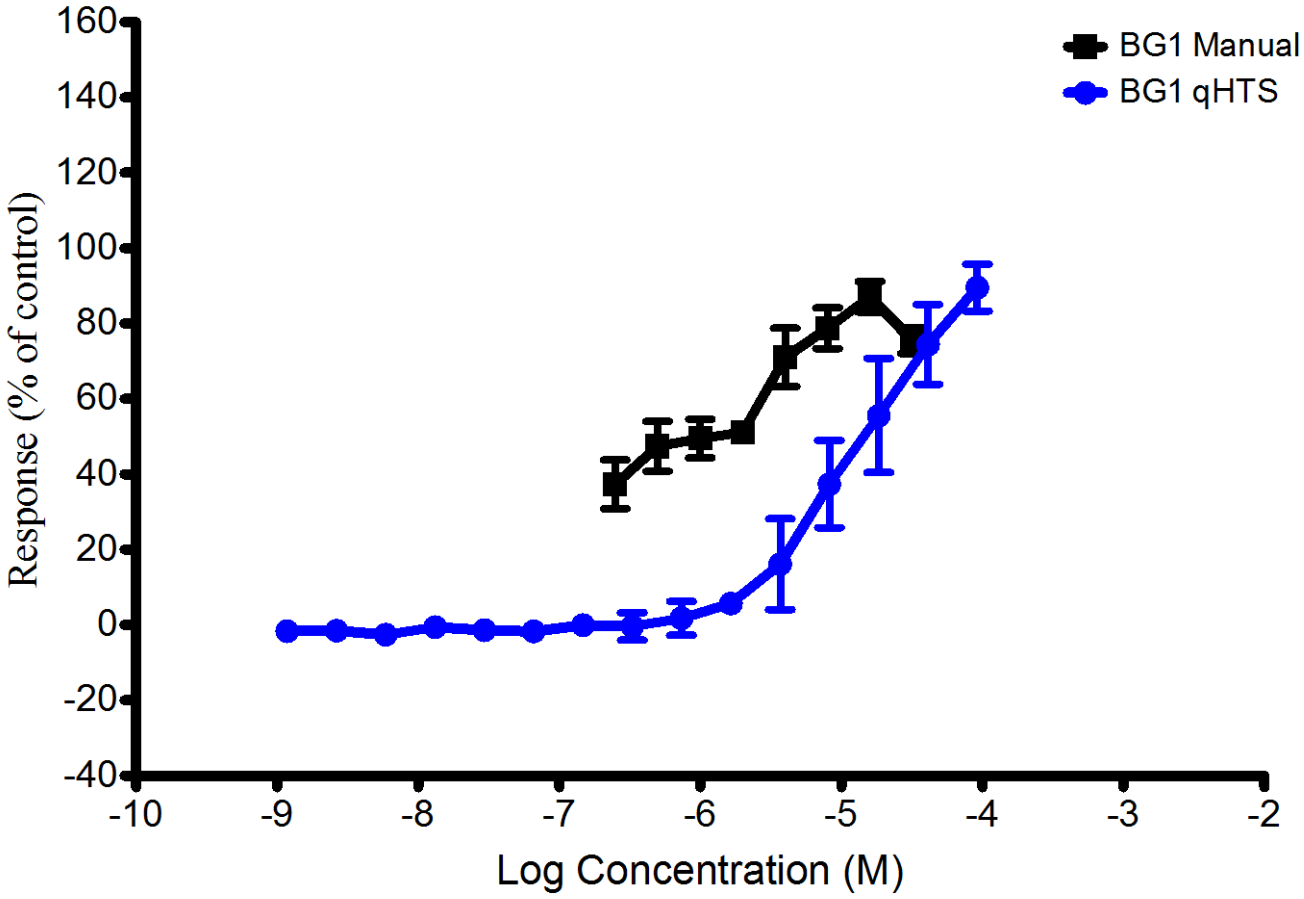
Bisphenol A


BG1 Manual
BG1 qHTS
Response (% of control)
160
140
120
100
80
60
40
20
0
-20
-40
-11
-10
-9
-8
-7
-6
-5
-4
-3
-2
Log Concentration (M)

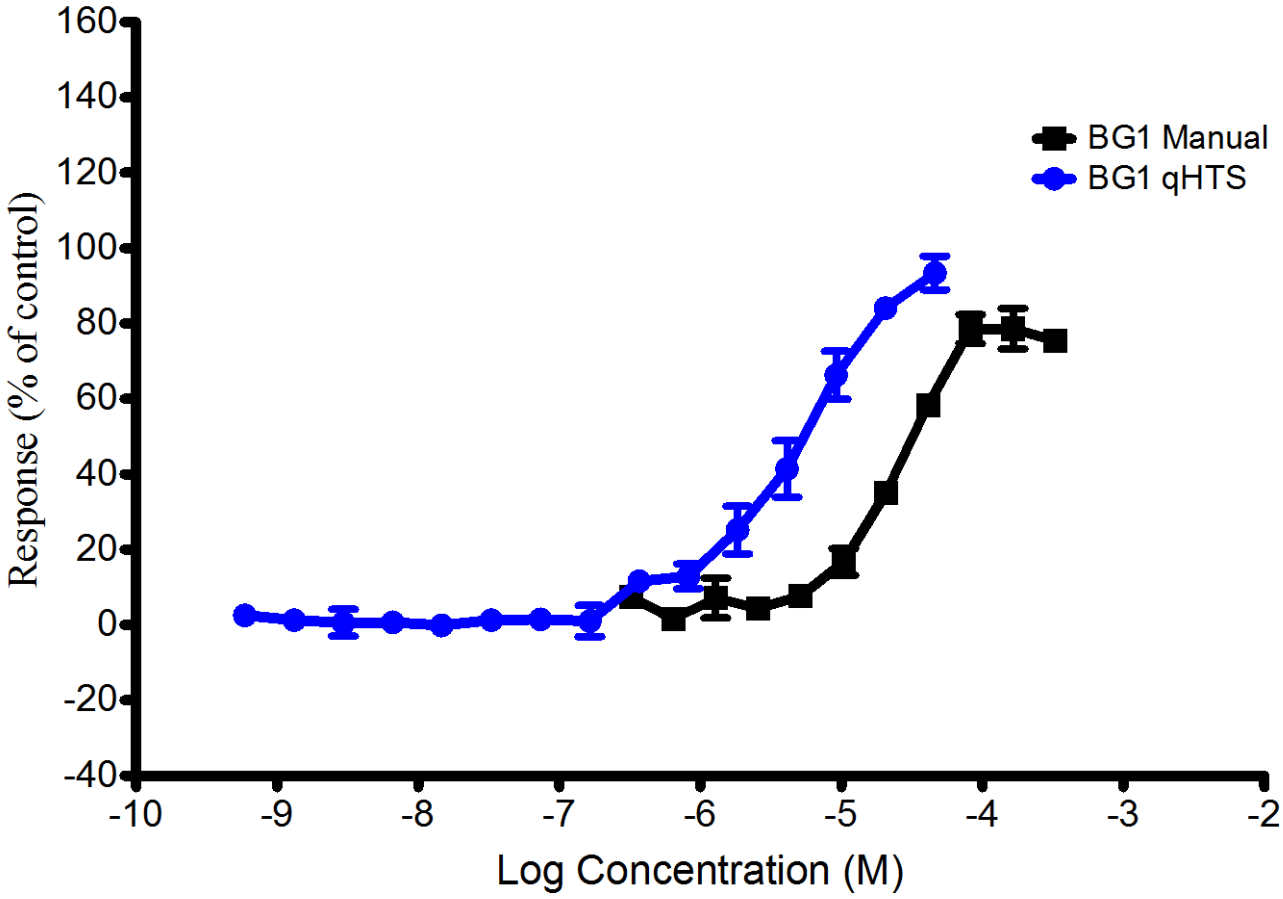
Apigenin


Response (% of control)
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
BG1 Manual
BG1 qHTS
-10
-9
-8
-7
-6
-5
-4
-3
Log Concentration (M)

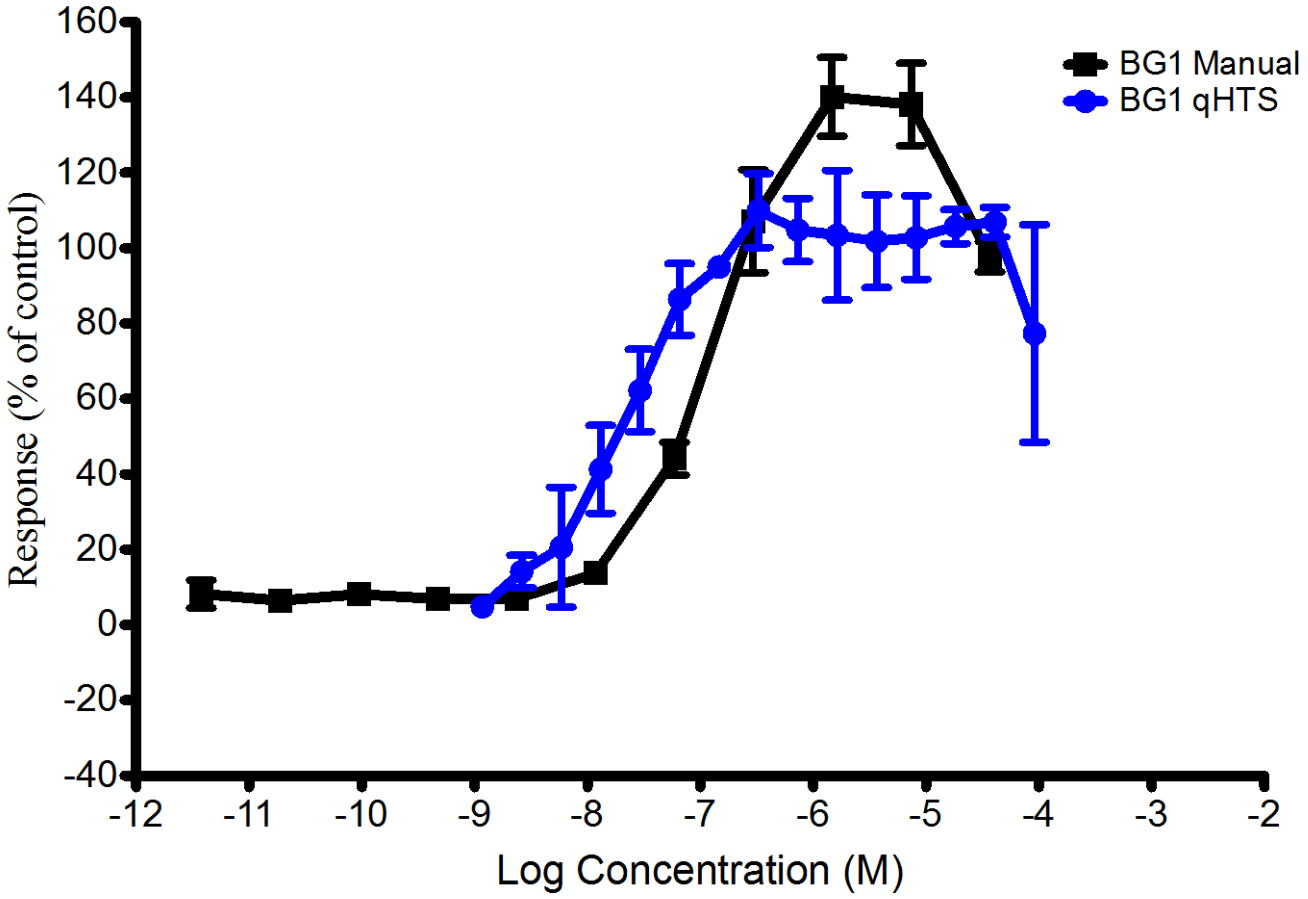
Butylbenzyl phthalate



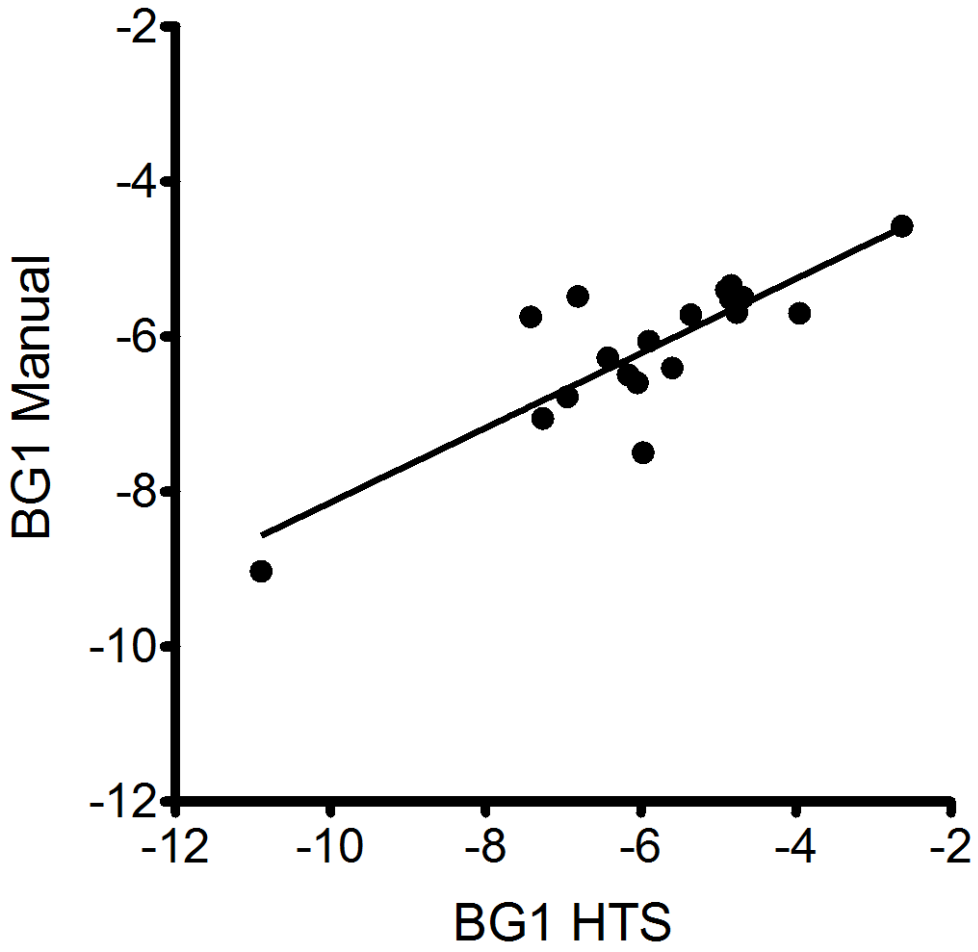
4-Hydroxyandrostenedione



17β-Trenbolone

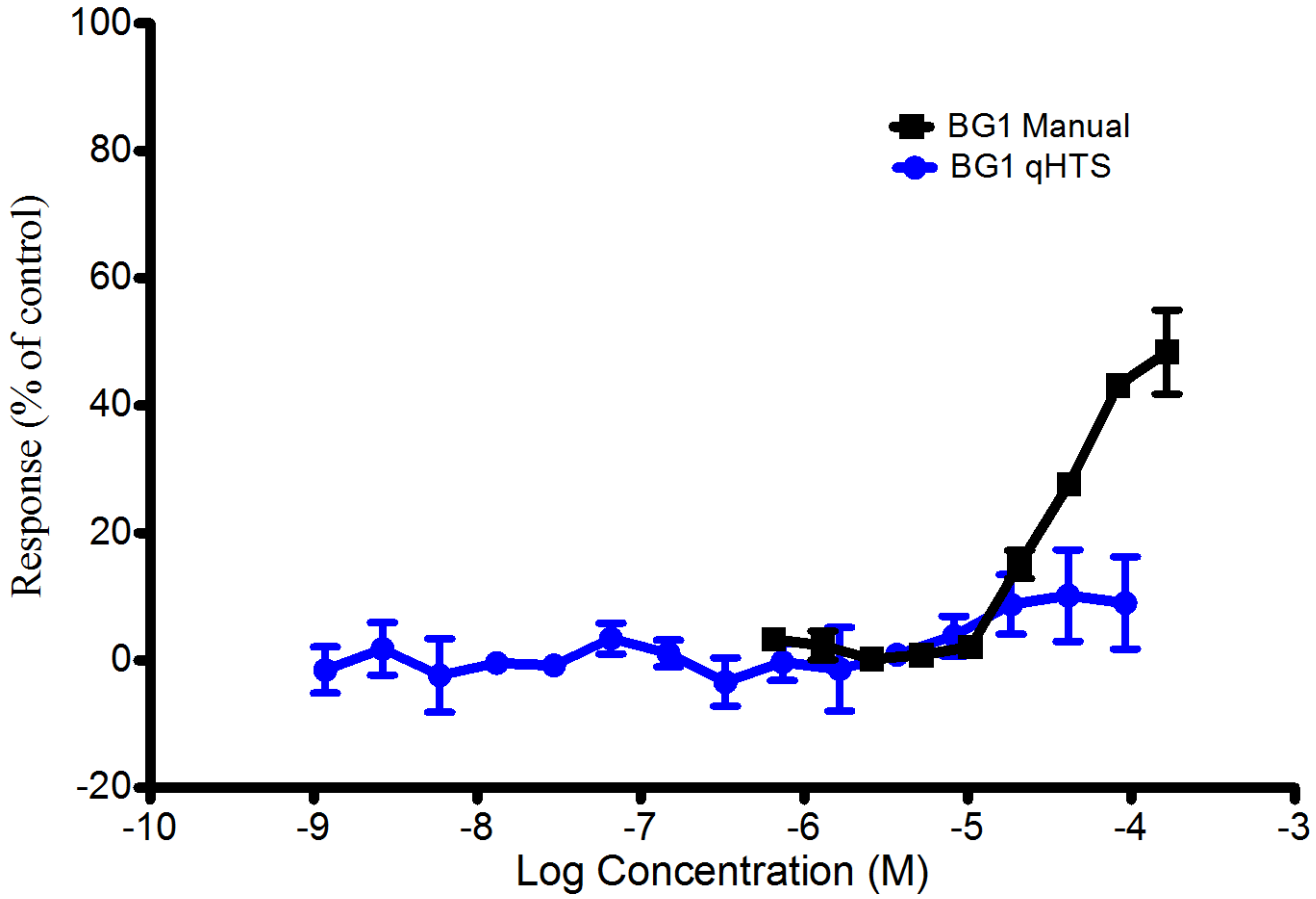


Comparison of BG1 Manual and qHTS EC_{50} Values (Agonist)

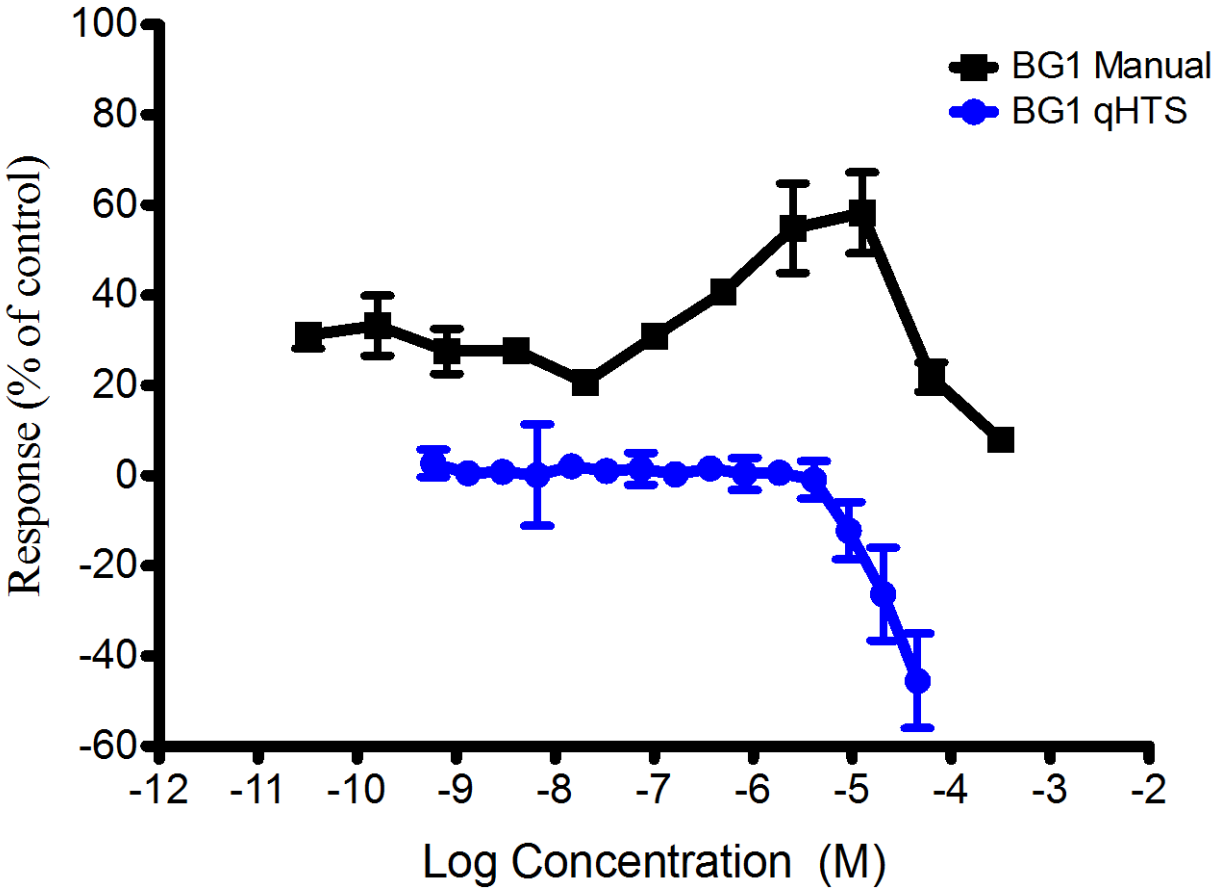


Slope of linear regression line is 0.48, r^2 is 0.69

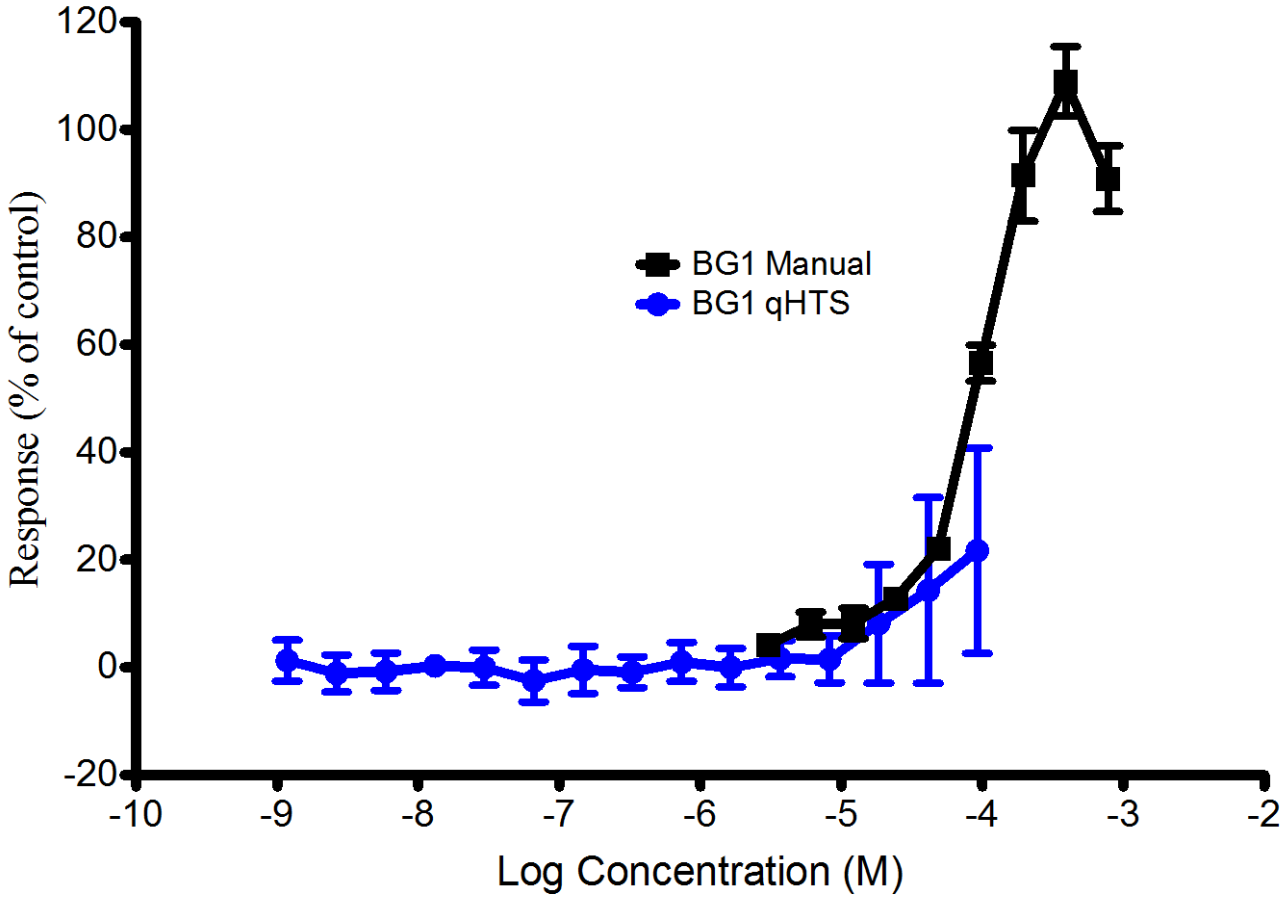
2-*sec*-butyl phenol



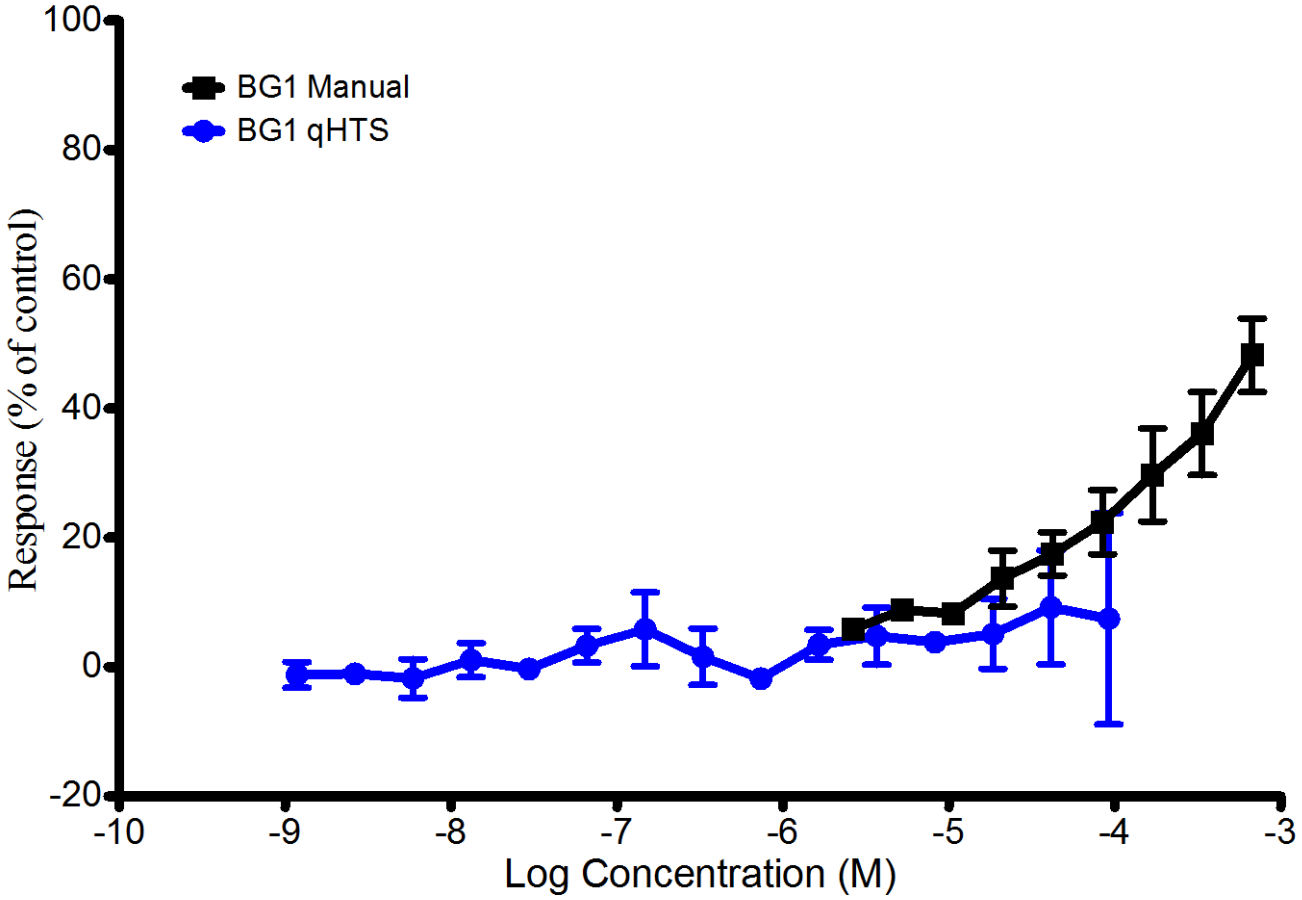
Nilutamide


BG1 Manual
BG1 qHTS
Response (% of control)
100
80
60
40
20
0
-20
-40
-60
-12
-11
-10
-9
-8
-7
-6
-5
-4
-3
-2
Log Concentration (M)

Phenolphthalin



Dicofol


BG1 Manual
BG1 qHTS
Response (% of control)
100
80
60
40
20
0
-20
-10
-9
-8
-7
-6
-5
-4
-3
Log Concentration (M)

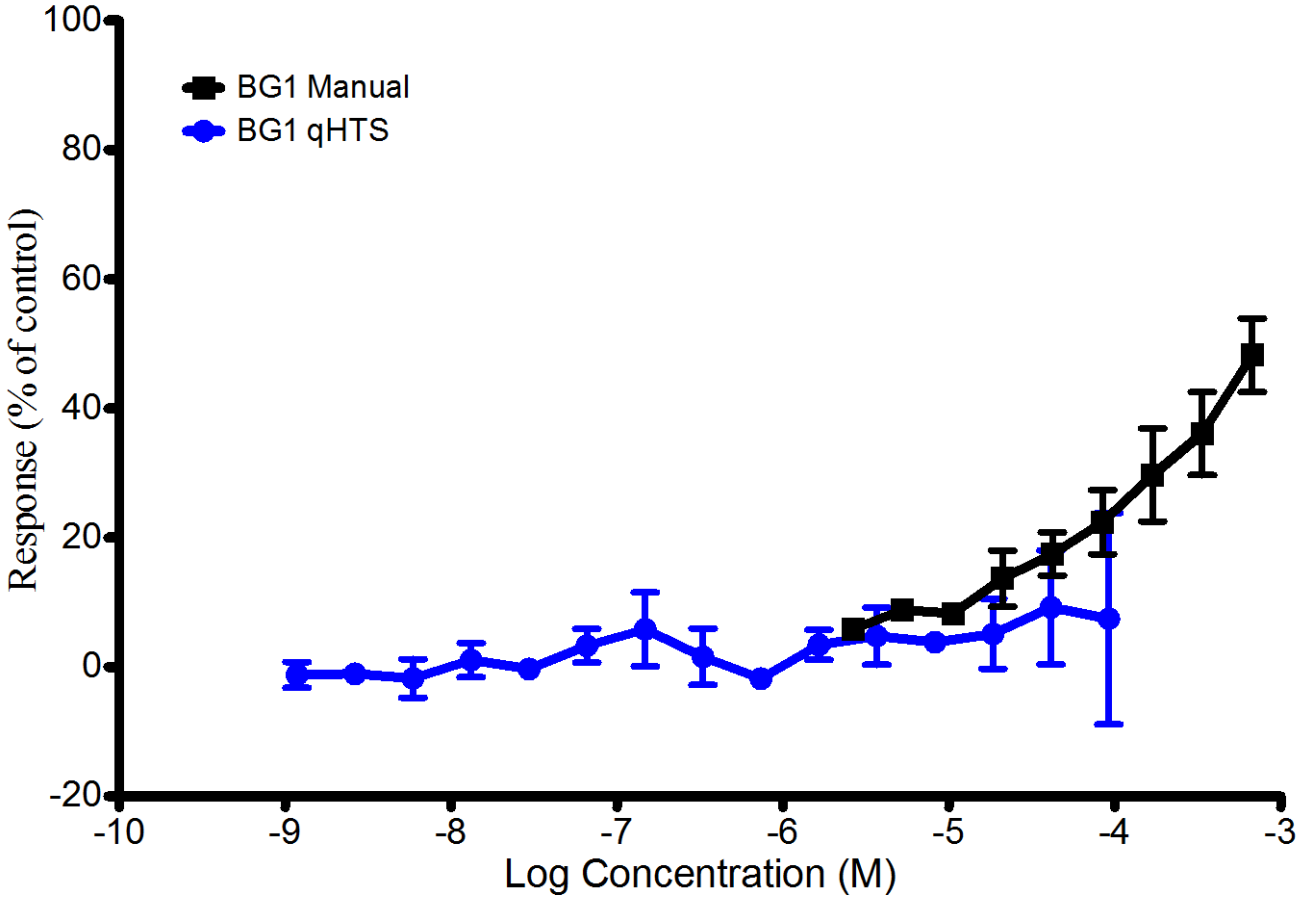
Agonist Accuracy

- Agonist Accuracy was calculated for 34 reference substances (27 positive, 7 negative)

correct

Ref Substance	BG1 Validation	BG1 qHTS
Positive (27)	27	26
Negative (7)	7	7
Overall	34/34	33/34

Dicofol


BG1 Manual
BG1 qHTS
Response (% of control)
100
80
60
40
20
0
-20
-10
-9
-8
-7
-6
-5
-4
-3
Log Concentration (M)

ICCVAM Recommendations, BG1 TMER:

- “As ER antagonists are identified, additional studies/evaluations may be conducted to expand the database of positive substances tested and thereby better characterize the usefulness and limitations of the BG1Luc ER TA test method as a screening test to identify substances with ER antagonist activity. “

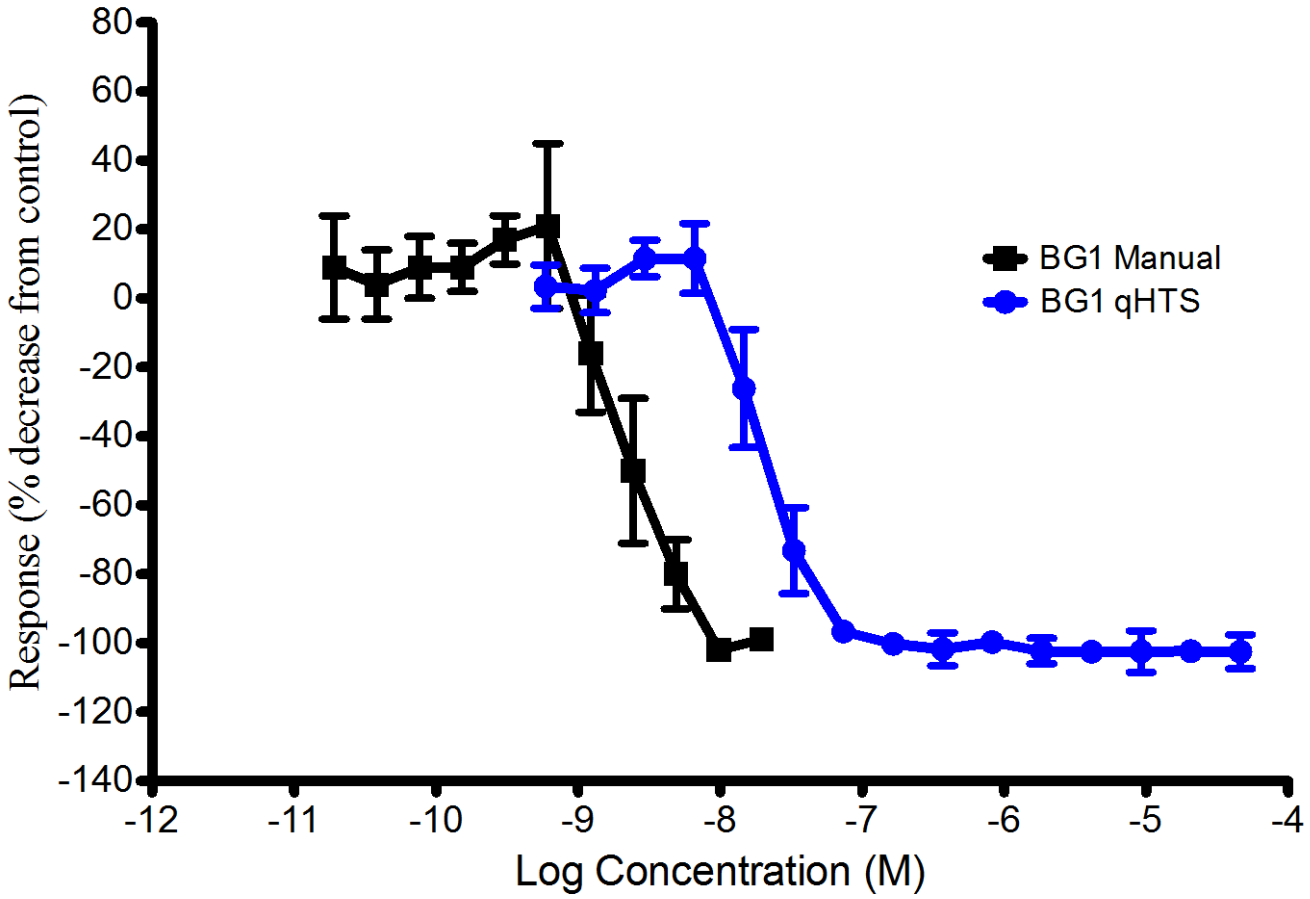
Antagonist Concordance

- Antagonist Concordance was calculated for 73 substances

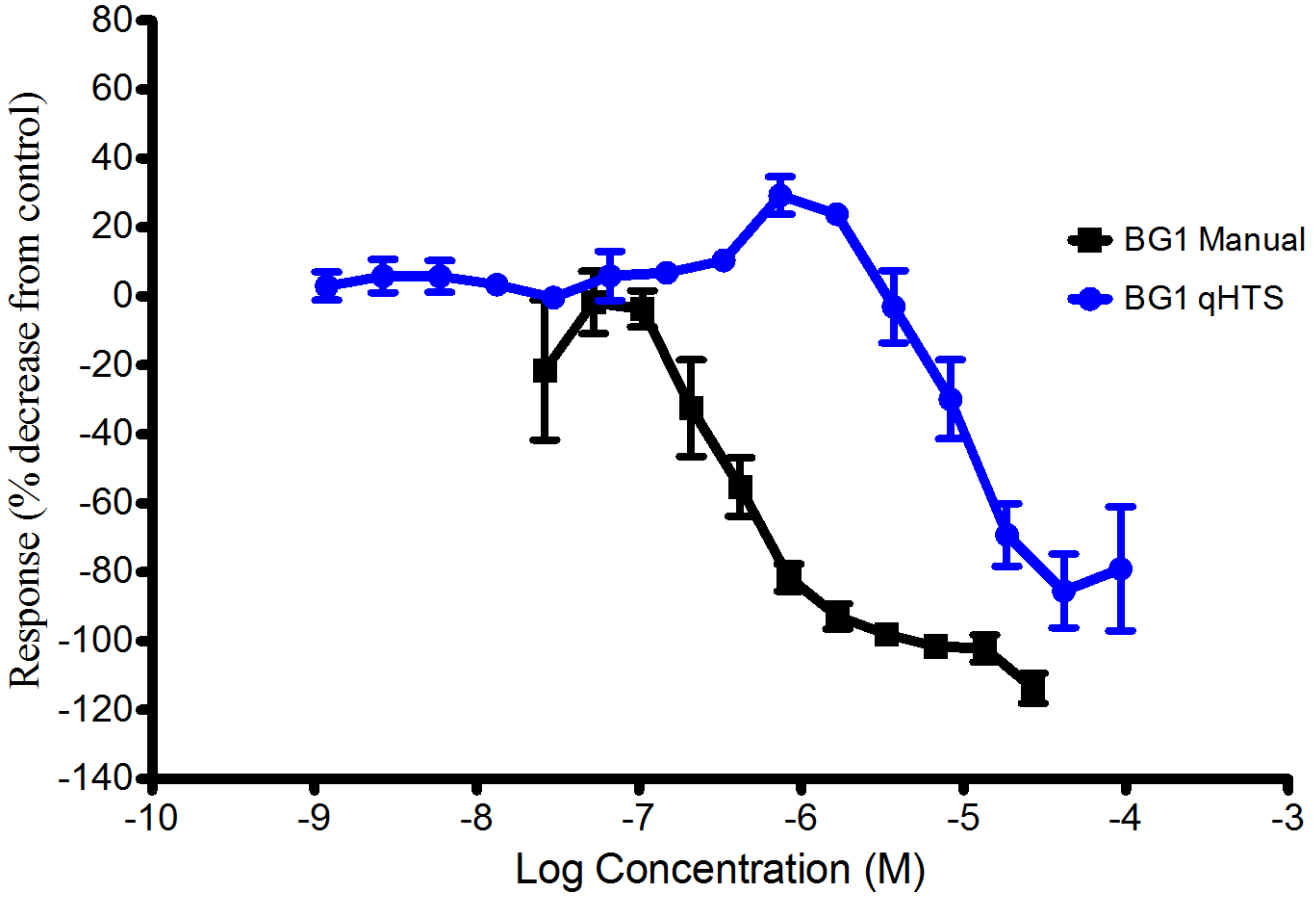
		BG1 qHTS Classification	
		Positive	Negative
BG1 Validation Classification	Positive	6	3
	Negative	0	64

- Concordance between the manual and qHTS assays was 96% (70/73)

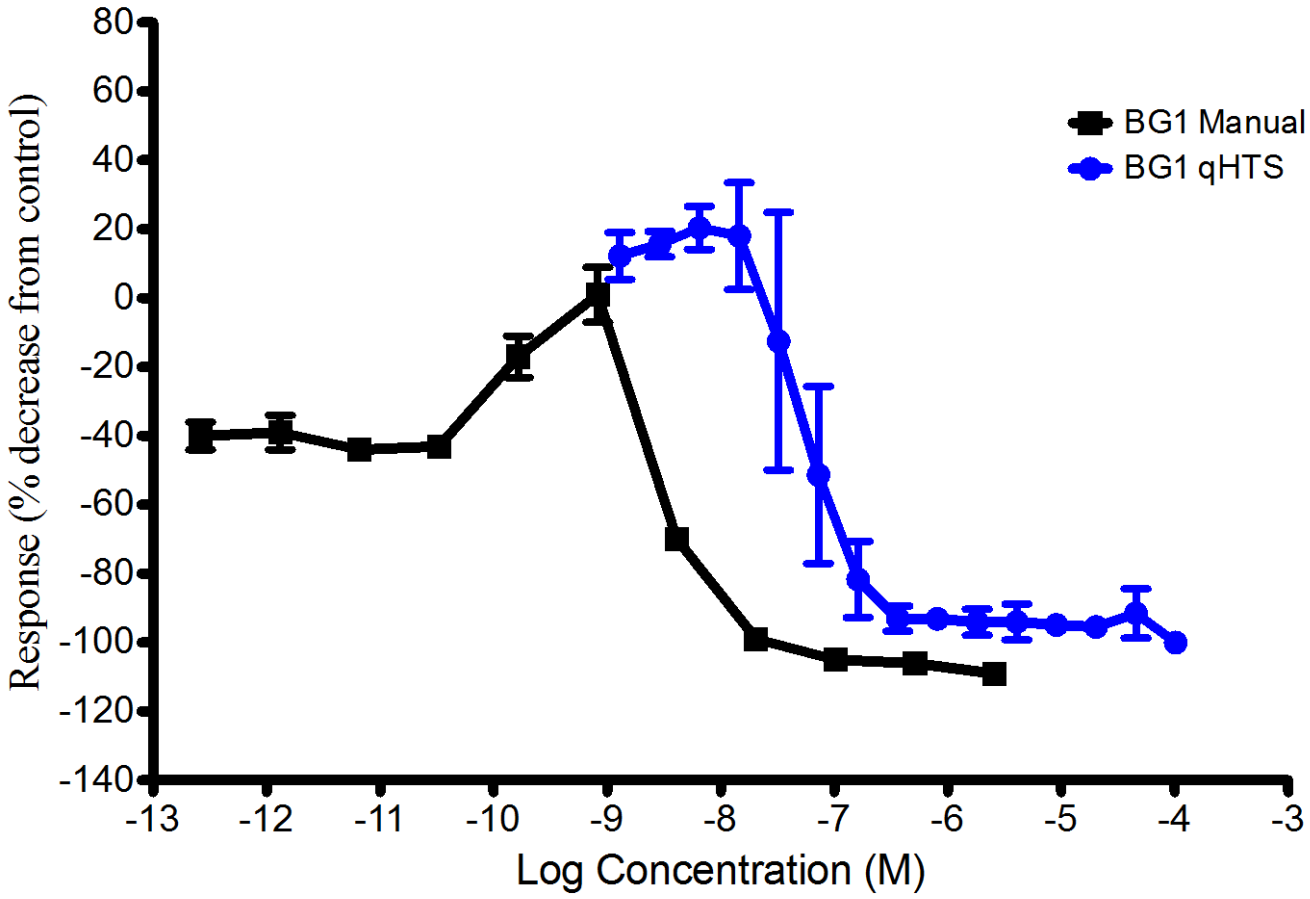
Raloxifene HCl



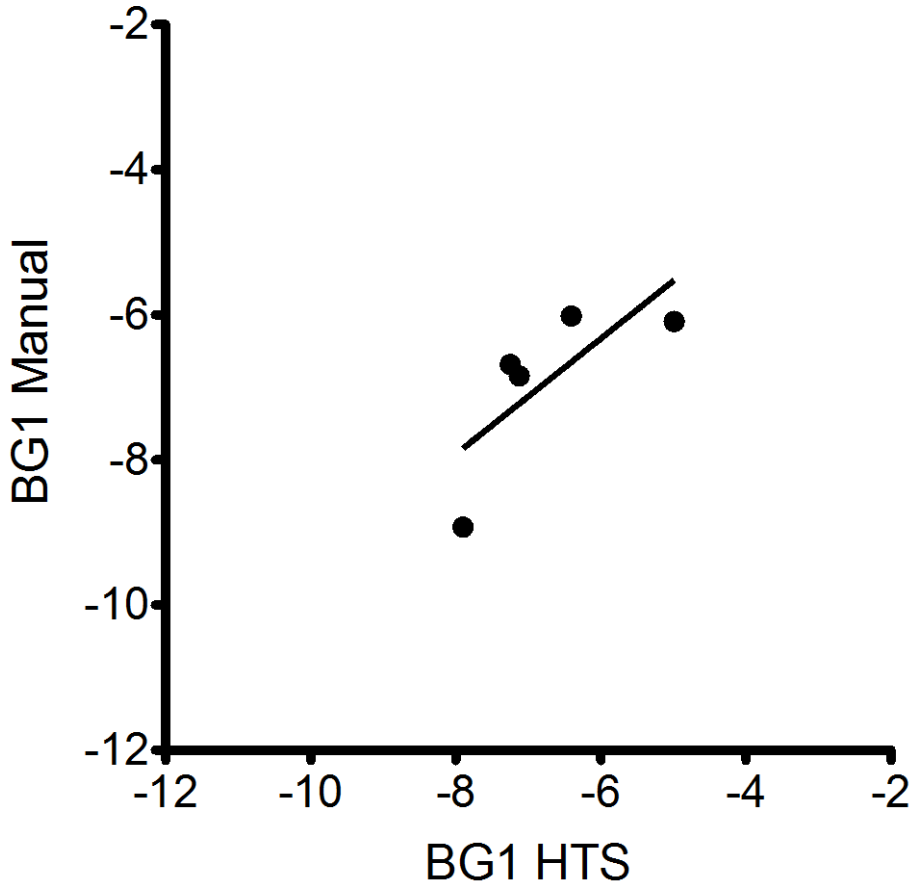
Tamoxifen


80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
Response (% decrease from control)
-10
-9
-8
-7
-6
-5
-4
-3
Log Concentration (M)
BG1 Manual
BG1 qHTS

4-Hydroxytamoxifen

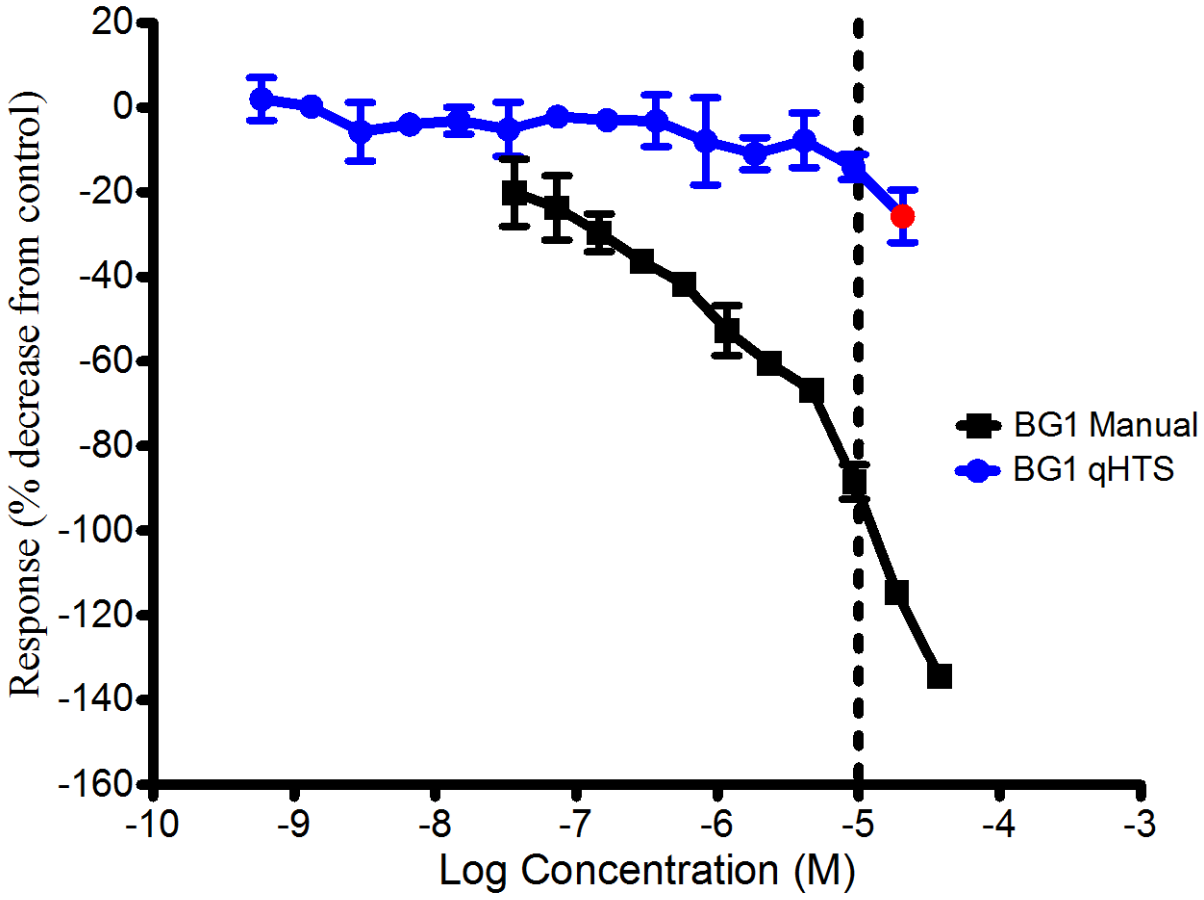


Comparison of BG1 Manual and qHTS IC_{50} Values (Antagonist)



Slope of linear regression line is 0.80, r^2 is 0.56

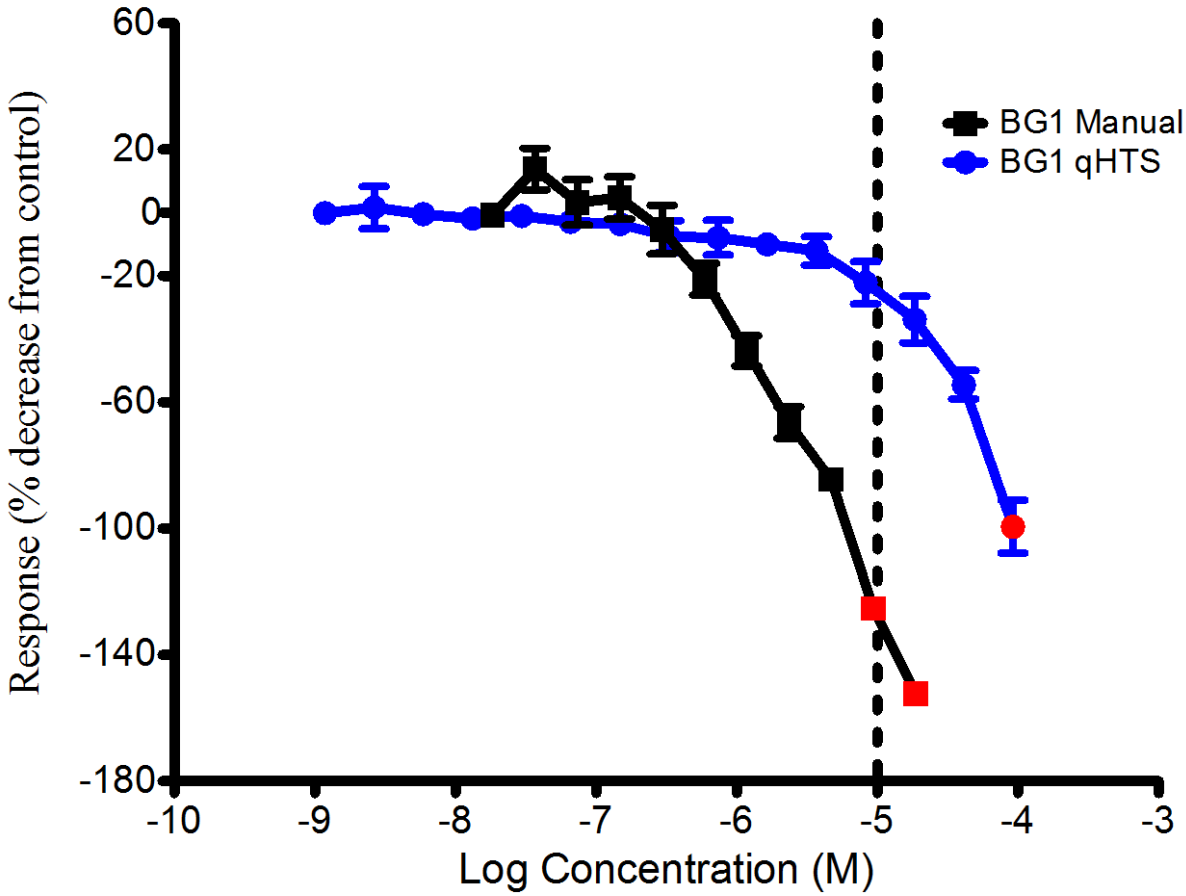
Apomorphine



Dashed line indicates the 10μM cutoff

Red dots in the concentrations at which cell viability was < 80% of controls

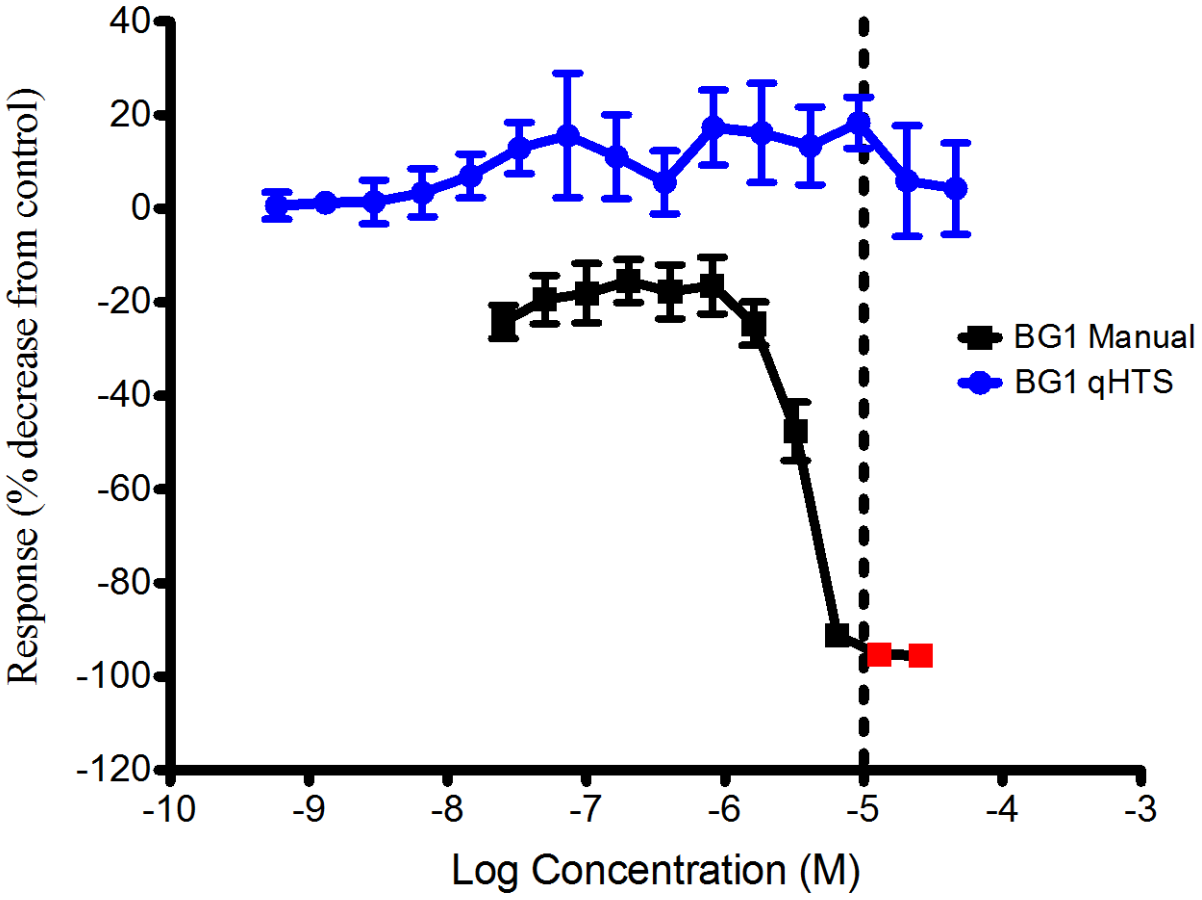
Ketoconazole



Dashed line indicates the 10μM cutoff

Red dots in the concentrations at which cell viability was < 80% of controls

Medroxyprogesterone acetate



Dashed line indicates the 10μM cutoff

Red dots in the concentrations at which cell viability was < 80% of controls

Antagonist Accuracy

- Antagonist Accuracy was calculated for 25 substances (3 positive, 22 negative)

correct

Ref Substance	BG1 Validation	BG1 qHTS
Positive (3)	3	3
Negative (22)	22	22
Overall	25/25	25/25

Conclusions

- Tox21 qHTS and manual testing using BG1 ER TA agonist and antagonist protocols produced almost identical results in terms of accuracy, and provided comparable AC50 values.

- The few discrepancies noted between Tox21 qHTS and the manual method appear to be related primarily to differences in the upper limit of testing concentrations
  - Tox21 qHTS uses an upper limit of 100 µM, whereas the manual method detected some “active” substances above this concentration

Next Steps

- Evaluate automated activity calling software for concordance with manual method (NTP, NCATS, EPA)

Next Steps

- Evaluate automated activity calling software for concordance with manual method (NTP, NCATS, EPA)
- Assess qHTS repeatability compared to manual method

ICCVAM Recommendations, BG1 TMER:

- Results from the BG1Luc ER TA test method were examined for concordance with published reports of ER binding for 34 reference substances. There was 97% (33/34) concordance between the BG1Luc ER TA test method and ER binding data from the literature, and 100% sensitivity (no false negatives)**. In light of the excellent degree of agreement between ER binding and BG1Luc ER TA data, it appears that evaluating results from BG1Luc ER TA agonist and antagonist testing may provide a viable alternative to conducting ER binding studies.**

Next Steps

- Evaluate automated activity calling software for concordance with manual method (NTP, NCATS, EPA)
- Assess qHTS repeatability compared to manual method
- NICEATM has identified 142 chemicals in the Tox21 10K library for which RUC ER binding data are also available (NCTR database)

ICCVAM Recommendations, BG1 TMER:

- Results from the BG1Luc ER TA test method were examined for concordance with published data from the uterotrophic bioassay (n = 13 reference substances), which is currently listed as part of the EDSP Tier 1 screening battery. There was 92% (12/13) concordance between the BG1Luc ER TA test method and the uterotrophic bioassay data, and 100% specificity (no false negatives). These data indicate that the BG1Luc ER TA agonist test method has very good agreement with the *in vivo* results obtained with the uterotrophic bioassay. **Accordingly, ICCVAM recommends that further work be carried out to determine if the BG1Luc ER TA test method could be used in combination with other methods (to include *in vitro* metabolic activation) in a weight-of-evidence approach to replace the uterotrophic bioassay.**

Next Steps

- Evaluate automated activity calling software for concordance with manual method (NTP, NCATS, EPA)
- Assess qHTS repeatability compared to manual method
- NICEATM has identified 142 chemicals in the Tox21 10K library for which RUC ER binding data are also available (NCTR database)
- NICEATM has identified 58 chemicals in the Tox21 10K library for which rat uterotrophic data are also available (CERI database)