Generation of a Bhas 42 Cell Transformation Assay Historical Database

Shannon W. Bruce, Michelle L. Klug LaForce, Sandra D. Springer, Amanda Fernandez
Inotiv, Rockville, MD USA

Abstract

The Bhas 42 cell transformation assay (CTA) is a sensitive short-term system for predicting chemical carcinogenicity. Bhas 42 cells were established from BALB/c 3T3 cells by the transfection of v-Ha-*ras* gene and postulated to be initiated in the two-stage carcinogenesis theory. The Bhas 42 CTA measures the induction of morphologically transformed (MT) foci that show invasive growth into the monolayer of surrounding contact-inhibited cells. The assay protocol consists of two parts, the initiator assay (Sasaki, *et.al.* 1988, 1990, Asada, *et.al.* 2005) and promoter assay (Ohmori, *et.al.* 2004, 2005*)* to detect tumor-initiating and promoting activity, respectively, of chemical carcinogens. The positive control used for the initiator arm is 3-methylcholanthrene (3MCA) at 1.0 µg/mL and for the promoter arm is 12-*O*-tetradecanoylphorbol-13-acetate (TPA) at 50 ng/mL. Vehicle controls selected for database were deionized water, dimethyl sulfoxide (DMSO) and ethanol. In the Bhas 42 assay initiator arm, the mean MT foci values are 5.17, 5.24, and 4.53 for the vehicle controls (aqueous, dimethyl sulfoxide, and ethanol) and 16.17 for the positive control. In the Bhas 42 assay promoter arm, the mean MT foci values are 5.25, 6.67, and 5.63 for the vehicle controls (aqueous, dimethyl sulfoxide, and ethanol) and 31.23 for the positive control. Thus, we have established a historical database for the most commonly used vehicles and positive control along with 95% control limits for both the initiator and promoter arms of the Bhas 42 CTA.

Methods

Bhas 42 cells (v-Ha-*ras*-transfected BALB/c 3T3 clone A31-1-1 cells) free from bacteria, fungi and mycoplasma were purchased from JCRB Cell Bank, (National Institute of Biomedical Innovation (NIBIO, Osaka, Japan)). Cells are cultured in an incubator under standard conditions (5±1% CO_2 at 37±1ºC with ≥85% humidity). The cells were cultivated in Eagle's Minimum Essential Medium (Gibco, Grand Island, NY, USA), supplemented with 100 units/mL of penicillin and 100 µg/mL of streptomycin (Gibco) and 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA) (M10F). The cells were sub-cultured using 0.25% trypsin-EDTA (Sigma Aldrich, St. Louis, MO, USA) to maintain a sub-confluent state. Within two passages after thawing, the cultured cells were suspended at 5×10^5 cells/mL in fresh M10F containing 5% dimethyl sulfoxide (DMSO, Sigma Aldrich), frozen in 1.0 mL volumes at −80ºC and then stored in liquid nitrogen. Cells are started from ampule for each experiment performed.

The vehicles used in both initiator and promoter assays were dimethyl sulfoxide (DMSO; Sigma Aldrich), ethanol (EtOH; Sigma Aldrich) and Phosphate Buffered Saline without calcium and magnesium (PBS; Gibco). The vehicles were tested at 0.1% (EtOH), 0.2% (DMSO), 0.5% (DMSO), and 5% (PBS). The positive control for the initiator assay was 3-methylcholanthrene (3MCA; Sigma Aldrich) dosed at 1.0 mg/mL in 0.2% DMSO . The positive control for the promoter assay was 12-*O*-tetradecanoylphorbol-13-acetate (TPA; Sigma Aldrich) dosed at 50 ng/mL in 0.2% DMSO. Dulbecco's modified Eagle's medium/Ham's F12 (Gibco) supplemented with 100 units/mL of penicillin and 100 µg/mL of streptomycin (Sigma Aldrich) and 5% FBS (HyClone) (DF5F) was used to prepare dose formulations for the transformation assays. All vehicle and positive control dose formulations were prepared fresh on each dosing day just prior to use. All assays were performed in 6-well plates. Four different technicians performed the assays which comprise the database.

Results

The average number of foci observed for each vehicle tested was less than 10 or 12 foci per well per guidance document for initiator and promoter assays, respectively. The positive controls were significantly increased when compared to the concurrent vehicle controls. Historical control data values are presented in the tables below.

Conclusions

In the cell transformation assays as in genetic toxicology assays, historical data play an important role in evaluating the validity of a test, as well as in interpreting the results for test materials. Thus, robust historical databases are important for these assays. Our data show that consistent results can be obtained from the Bhas 42 cell transformation assay across assay performance by different technicians. Additionally, a historical database has been established for the performance of the Bhas 42 cell transformation assay using the most common vehicle and positive controls.

References

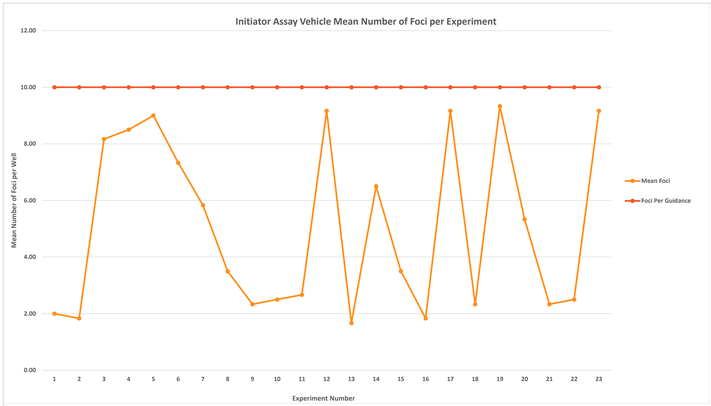
Asada, S., Sasaki, K., Tanaka, N., Hayashi, M., Umeda, M. (2005). Detection of initiating activities of chemicals using v-Ha-ras-transfected BALB/c 3T3 cells (Bhas 42 cells), Mutat. Res. 588 7-21.

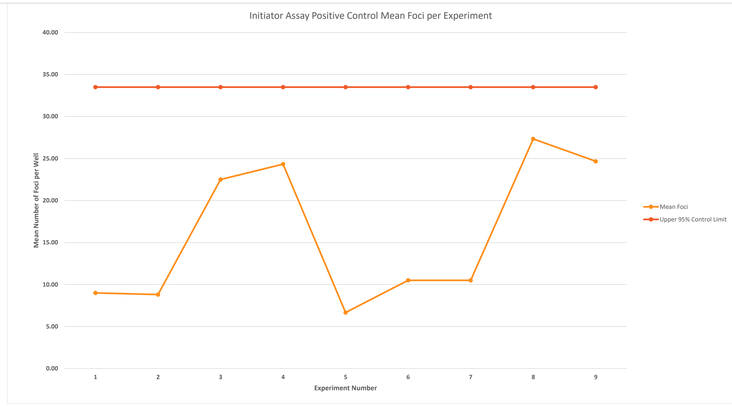
Guidance Document On the In Vitro Bhas 42 Cell Transformation Assay Series On Testing & Assessment No. 231, Env/Jm/Mono(2016)1 Organisation De Coopération et de Dévelopement Économiques Organisation for Economic Co-operation and Development (OECD) 20 July 2017.

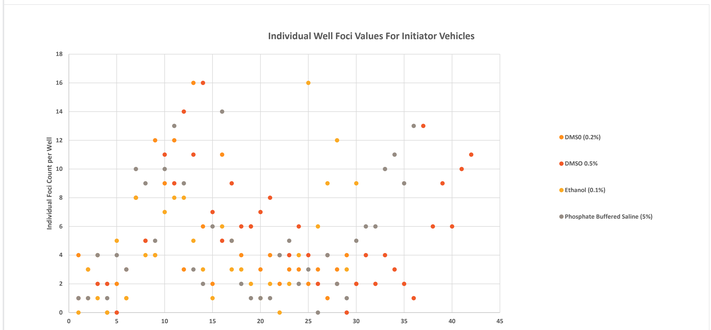
Ohmori, K., Sasaki, K., Asada, S., Tanaka, N., Umeda, M. (2004). An assay method for the prediction of tumor promoting potential of chemicals by the use of Bhas 42 cells, Mutat. Res. 557 191-202.

Sasaki, K., Mizusawa, H., Ishidate, M. (1988). Isolation and characterization of ras-transfected BALB/3T3 clone showing morphplogical transformation by 12-O-tetradecanoyo-phorbol-13-acetate, Jpn. J. Cancer Res. 79 921-930.

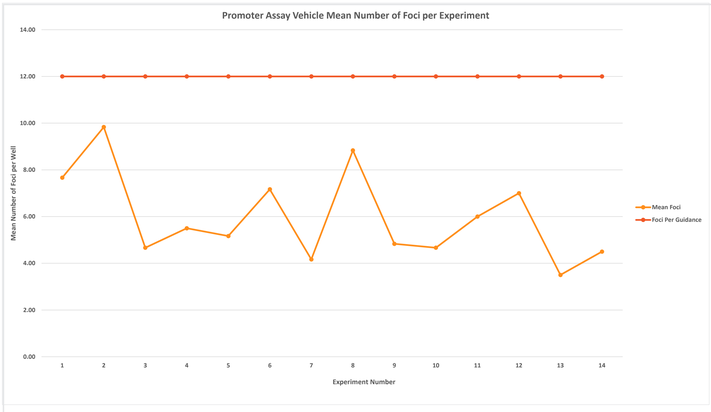
Sasaki, K., Mizusawa, H., Ishidate, M., Tanaka, N. (1990). Establishment of a highly reproducible transformation assay of a ras-transfected BALB/3T3 clone by treatment with promoters, Basic Life Sci. 52 411-416.

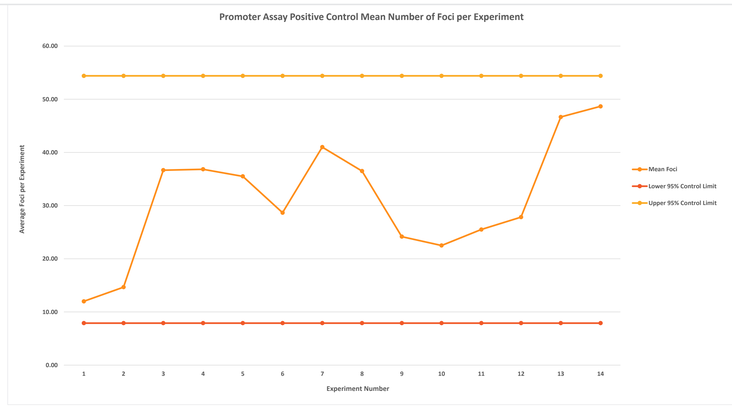


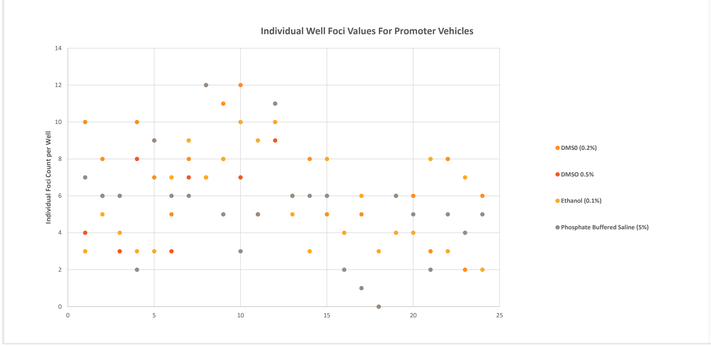




Initiator Assay	DMSO	Ethanol	Aqueous	Positive Control
Mean	5.24	4.53	5.17	16.29
Standard Deviation	3.88	3.79	4.00	8.60
95% Control Limit	0.00 – 12.99	0.00 – 12.12	0.00 – 13.16	0.00 – 33.49
Minimum/Maximum	0 – 16	0 – 16	0 - 14	1 - 33







Promoter Assay	DMSO	Ethanol	Aqueous	Positive Control
Mean	6.67	5.63	5.25	31.17
Standard Deviation	2.81	2.55	2.83	11.64
95% Control Limit	1.05 – 12.28	0.52 – 10.73	0.00 – 10.91	7.90 – 54.44
Minimum/Maximum	0 - 12	2 - 10	0 – 12	6 - 52