

The GADD45a-GFP (GreenScreen HC) Assay as a Validated Alternative Method: A Case Study

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Introduction

Method validity is defined by its relevance and reliability for a specific purpose and the VAM centres (ECVAM, ICCVAM, JaCVAM) have a critical role to play in expediting the roll-out of new alternatives. There is broad acknowledgement that the ICH battery of *in vitro* genotoxicity assays needs to be improved upon. One strategy is to consider new suitable methods as alternatives.

The GreenScreen HC (GSHC) assay meets all of the proposed requirements of a new test method. Published data reveal both its high sensitivity (genotoxic carcinogens & *in vivo* genotoxins) and high specificity (non-carcinogens & *in vivo* non-genotoxins). ECVAM operates a modular approach to the principles of test validity^[1,2]. Important facets of the 7 modules have already been performed for GSHC, independently of ECVAM, and these are considered here in preparation for a formal ECVAM review.

1: Test Definition: prediction of *in vivo* genotoxicity and genotoxic carcinogenicity

GSHC employs a GFP reporter of *GADD45a* transcription levels to create an indirect measure of genome damage. Transcription of *GADD45a* (Growth Arrest & DNA Damage) is up-regulated in response to numerous genotoxic stresses in different cell types – rapid, dose-dependent, conserved and largely p53-dependent. The reporter system is hosted in human, p53-competent, lymphoblastoid TK6 cells. There is a wealth of background literature in support of *GADD45a* – it was the only *GADD* gene to respond to ionizing radiation.

The 96-well format, 48h timeframe and accuracy of the results make GSHC highly adaptable in terms of its positioning as a tool in

drug discovery. The automation compatibility of GSHC lend its use to early screening strategies and compound profiling or lead optimisation.

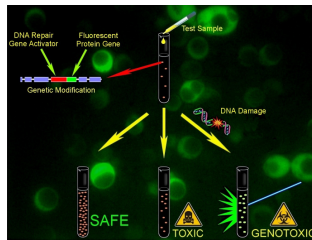


Figure 1. A schematic of the GSHC assay principle.

Numerous agencies have noted that safety assessment genotoxicity testing needs to be altered and improved. The high accuracy of GSHC may suggest a role in qualification of genotoxicity data from the *in vitro* regulatory battery. Ultimately, it could become an alternative method to the currently used *in vitro* mammalian genotoxicity tests. In addition, legislation such as REACH and the 7th Amendment to the European Cosmetics Directive are also driving the need for new, improved genotoxicity testing methods.

A definitive protocol SOP has been developed for each method (GSHC & GSHC S9). Test criteria and interpretation documents are also available.

2: Within-Laboratory Variability

Within-laboratory variability for GSHC was assessed in an initial validation study of 75 compounds performed by the originators^[3]. Compounds were tested 4 times by up to 4 different operators.

74 of 75 compounds generated the same genotoxicity outcome in all 4 replicate assays (53/54 single and 21/21 multiple operators).

Within-laboratory variability for GSHC S9 was assessed in an initial study of 56 compounds performed by the originators^[4]. Compounds were tested 3 times by up to 4 different operators.

48 of 56 compounds generated the same genotoxicity outcome in all 3 replicate assays.



Figure 2. Hastwell et al. (2006) [3]



Figure 3. Jagger & Tate et al. (2009) [4]

4: Between-Laboratory Variability

Elements of between-laboratory variability were built into the transferability study (see Module 3)^[5]. 16 coded compounds were assessed in quadruplicate at 4 independent sites: the originating centre, the initial transfer and optimisation site, a site with experience of the assay format and an inexperienced site. This study design was intended to more broadly expose areas of the assay protocol that required clarification or amendment and inform on the level of training necessary for naïve users, whilst providing indicative data of the variability of the assay with the SOP used both within and between laboratories.

- overall concordance with expected results = **92.5%**
- minor SOP improvements made

Table 1. Predictivity statistics (48h data) from the 4 sites.

	Site 1	Site 2	Site 3	Site 4	Overall
Sensitivity	100.0	86.4	100.0	92.8	94.0
Specificity	86.7	94.1	93.1	88.9	90.8
Predictive value (+)	83.3	95.0	93.9	88.5	91.8
Predictive value (-)	100.0	84.2	100.0	93.2	93.2
Concordance	87.5	89.7	96.2	95.4	92.5



Figure 4. Billinton et al. (2008) [5]

3: Transferability

A transferable method is one that can be demonstrated to be successfully repeated at a site other than the originating or optimising centre. Transferability is regarded as an important criterion in assessing the practicability of a new assay or method, and the reasons for this are two-fold:

- Determination of the degree of training required for successful transfer to an inexperienced centre or new user
- Identification of potential sources of both within-laboratory and between-laboratory variation.

Elements of a between-laboratory variability study were incorporated for GSHC (see Module 4)^[5].

GSHC transferred effectively to new laboratories:

- Materials and practical demonstrations were sufficient and cells were successfully revived and cultured

The GSHC S9 transferability manuscript is currently in preparation.

5: Predictive Capacity

Genotoxic carcinogenicity: sensitivity **95%**, specificity **87.5%** (109 cmpds)

Genotoxicity (*in vivo*): sensitivity **78%**, specificity **94%** (131 cmpds)

6 & 7 : Applicability Domain, Performance Standards

GSHC & GSHC S9 are applied in early screening strategies in compound profiling, hit-to-lead, lead optimisation and candidate selection. Performance standards, acceptance criteria and benchmarks continue to be assessed.

Conclusion

Evidence is accumulating in support of the GSHC assay as an alternative *in vitro* mammalian genotoxicity method. A 'presubmission' dossier has been submitted to ECVAM for evaluation.

References

- [1] Hartung et al., ATLA (2004) **32**:467-472
- [2] Hoffmann et al., ATLA (2008) **36**:343-352
- [3] Hastwell et al., Mutat. Res. (2006) **607**:160-175
- [4] Jagger & Tate et al., Mutagenesis (2009) **24**:35-50
- [5] Billinton et al., Mutat. Res. (2008) **653**:23-33