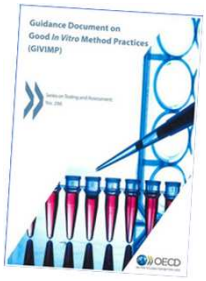
Good In Vitro Method Practices, a tool to increase test readiness for regulatory use

Ingrid Langezaal

Brussels, 27th of March 2023

1

Good in vitro method practice guidance (GIVIMP)



- Developed by OECD
- Guidance for the ***development***, ***use*** and ***implementation*** of *in vitro* methods

Actors of the Italian Commedia dell'Arte by Jean-Antoine Watteau

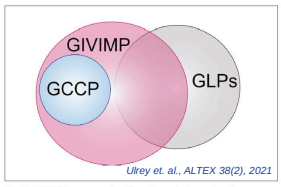
The 10 sections of GIVIMP:

1. Roles and responsibilities
2. Quality considerations
3. Facilities
4. Apparatus, material and reagents
5. Test systems
6. Test and reference/control items
7. Standard operating procedures (SOPs)
8. Performance of the method
9. Reporting of results
10. Storage and retention of records and materials

2

GIVIMP OECD Guidance Document on Good *In Vitro* Method Practices

GIVIMP is a tool that helps to implement good practices early in the method development process



Ulrey et. al., ALTEX 38(2), 2021

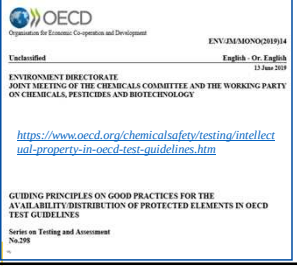
Fig. 1: GIVIMP incorporates the relevant elements of the GLPs and GCCP, however not all recommendations are applicable to every test method, developer or laboratory

- Improved efficiency of method development
- Increased reliability and integrity of generated data
- Methods are more easily transferred to others (e.g. for validation)
- **Results are more easily accepted for regulatory use**

3

General GIVIMP guidance:

- Ensure that all elements of the method are available to others so that results can be reproduced **(1.1)**



OECD
Organisation for Economic Co-operation and Development

ENV/JM/MONO(2019)14

Unclassified — English - Or. English — 13 June 2019

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

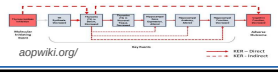
https://www.oecd.org/chemicalsafety/testing/intellectual-property-in-oecd-test-guidelines.htm

GUIDING PRINCIPLES ON GOOD PRACTICES FOR THE AVAILABILITY/DISTRIBUTION OF PROTECTED ELEMENTS IN OECD TEST GUIDELINES

Series on Testing and Assessment
No.298

- Control the quality of method components such as test system, material and reagents and verify batch to batch variation **(2.3 – 2.5)**
- Ensure retention of key records and documentation needed for transfer of the method to others **(10.1)**

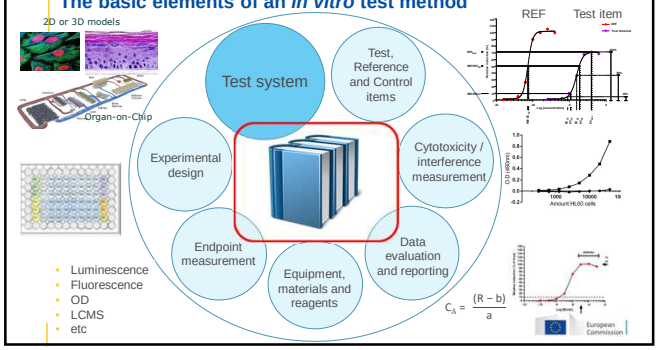
- Describe the mechanistic information obtained with the method + link with adverse outcome **(9.4)**



aopwiki.org/

4

The basic elements of an *in vitro* test method

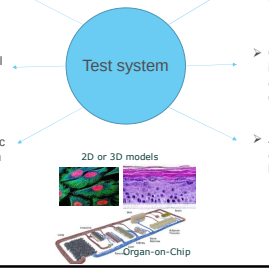


- Luminescence
- Fluorescence
- OD
- LCMS
- etc

5

GIVIMP guidance for the test system

- Importance of metabolic competence **(5.9)**
- Use reference and control items to confirm activity **(6.1)**
- Description of mechanistic information obtained from the test system **(9.4)**



- Apply Good Cell Culture practice (**Annexes A and B**)
- Quality control: Confirm identity, functionality, purity and absence of contamination **(2.4 & 5.8)**
- Appropriate maintenance and handling **(5.4)** and cell banking **(5.5)**

6

GIVIMP guidance for the experimental design

- Identify the samples & controls to be tested and the number of replicates (**8.2**)
- Define acceptance criteria for all critical components and aspects of the method based on historical data.(**8.1**)
- Design a plate layout / experimental setup (**8.2.1**)

Experimental design

Example plate layout:

	2	3	4	5	6	7	8	9	10	11
B	SC	RI-C1	RI-C2	RI-C3	RI-C4	RI-C5	RI-C6	RI-C7	RI-C8	PC
C	SC	RI-C1	RI-C2	RI-C3	RI-C4	RI-C5	RI-C6	RI-C7	RI-C8	PC
D	SC	RI-C1	RI-C2	RI-C3	RI-C4	RI-C5	RI-C6	RI-C7	RI-C8	PC
E	SC	TH-C1	TH-C2	TH-C3	TH-C4	TH-C5	TH-C6	TH-C7	TH-C8	NC
F	SC	TH-C1	TH-C2	TH-C3	TH-C4	TH-C5	TH-C6	TH-C7	TH-C8	NC
G	SC	TH-C1	TH-C2	TH-C3	TH-C4	TH-C5	TH-C6	TH-C7	TH-C8	NC

7

GIVIMP guidance for equipment, materials and reagents

Equipment, materials and reagents

- Ensure that equipment is maintained, calibrated and validated (**4.1**)
- Retain information from materials and reagents: supplier, catalogue and batch numbers, preparation steps etc. (**4.2**)
- Avoid the use of serum and animal-derived ingredients (**4.3**)
- Avoid the use of antibiotics (**4.4**)

Bartnicka et. al. 2021
https://publications.jrc.ec.europa.eu/repository/handle/JRC125904

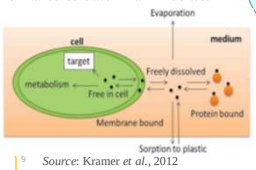
8

GIVIMP guidance for the test, reference and control items

- Be aware of biokinetics features. Avoid volatile, poorly soluble and unstable chemicals as controls (**6.9**)
- Select appropriate reference and control items, relevant for the mode of action measured (**6.1**)
- Appropriately prepare the test item and generate dose-response information where possible (**6.3 & 6.4**)
- Perform characterisation: composition, purity, homogeneity, phys-chem properties, solubility, stability under assay conditions (**6 & 6.5**)

Test, Reference and Control items

Schematic representation of some processes that can cause the final target concentration to be different than the nominal concentration in an *in vitro* test

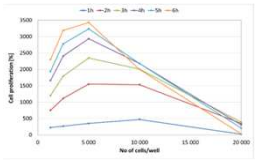


Source: Kramer *et al.*, 2012

9

GIVIMP guidance for the endpoint measurement

- Perform in-house validation of the measurement endpoint(s) (**8.3**)
  - Detection limits / cut off values
  - Linearity and dynamic range
  - Within and between run precision
  - Sensitivity
  - Specificity
  - Reproducibility within and between runs



Example of optimisation experiment, where % cell proliferation of GH3 cells was measured after 1, 2, 3, 4, 5 or 6h incubation with alamar blue.

Endpoint measurement

- Luminescence
- Fluorescence
- OD
- LCMS
- etc.

10

GIVIMP guidance for the cytotoxicity / interference measurement

- Test for interference of the test item with the test system (**6.10**) under the same conditions as the main endpoint.
- Test for interference of the test item with the test method (**6.11**) under the same conditions as the main endpoint.

Cytotoxicity / interference measurement

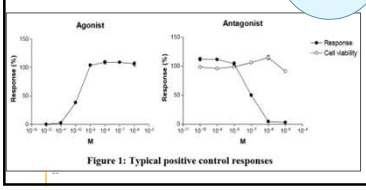


Figure 1: Typical positive control responses

Possible interference	Control to check
Degradation of reagents (e.g. luciferin, MTT, resazurin)	Test item + detection reagent, without test system
Similar properties to the endpoint (OD, fluorescence)	Test item only
Loss of analyte in an analytical method	Spike the samples with a control analyte to quantify.

11

GIVIMP guidance for the data evaluation and reporting

- Define and describe the data analysis to be performed (**8.2.2**)
  - Transformation of data using the reference item results.
  - Calculate parameters (e.g. EC50/IC50, % activity)
  - Provide calculations
- Ensure Data Integrity, FAIR principles (**10.1**)
- Make use of publicly available repositories.

Data evaluation and reporting

Transparency
The Transparency and Openness Promotion (TOP) guidelines from the Open Science Framework https://osf.io/ud578/

How OSF supports your research

12

When is a test method ready to be considered for regulatory use?

1) The method was developed using good scientific and quality practices.
2) All elements of the *in vitro* method are well described and procedures on how to perform the method are available in (a set of) SOP(s)
3) The method is in-house validated and reproducible results can be obtained with the reference and control chemicals.
4) All elements of the *in vitro* method are available to others.
5) The method is relevant for adverse outcome in humans and/or the environment.

Phase I	Max. score
1 Test system	9
2 Exposure scheme	3
3 Documentation/SOP	3
4 Main endpoints	4
5 Cytotoxicity	4.5
6 Test method controls	5
7 Data evaluation	7
Sum	**35.5**

Phase I		
Score	**Grading**	**Explanation of grading**
0-7	D	Test results invalid
8-17	C	Substantial improvements required to be ready
18-28	B	Improvements required to be ready
29-35.5	A	Test method is close to ready or ready

Example test readiness scoring system, developed by EURL ECVAM on basis of Bal-Price et. al. 2018

6) Transfer the method to others to reproduce results.
7) Validation for regulatory purpose.

13

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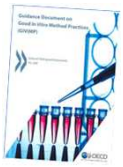
Ingrid.LANGEZAAL@ec.europa.eu



Available on OECD e-Library
https://doi.org/10.1787/20777876

Also available on the
OECD Series for Testing and Assessment No. 286

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https://etplas.eu/learn/

EU-60: Developing in vitro methods and approaches for scientific and regulatory use

14