The European Commission's Science and Knowledge Service

Joint Research Centre

Good *In Vitro* Method Practices (GIVIMP)

Sandra Coecke and Gerard Bowe

Improving Science, Advancing Animal Welfare :Towards better In Vitro Practices
Glorieuxpark, Eindhoven, NL Utrecht University Campus, The Netherlands
1st of June, 2018

European Commission

In vitro method development safeguarding scientific integrity and quality

GIVIMP

GUIDANCE DOCUMENT ON GOOD *IN VITRO* METHOD PRACTICES

Trusted by decision makers

Used by industry

European Commission

GIVIMP

is a response to a more general "crisis in reproducibility" in scientific data generation

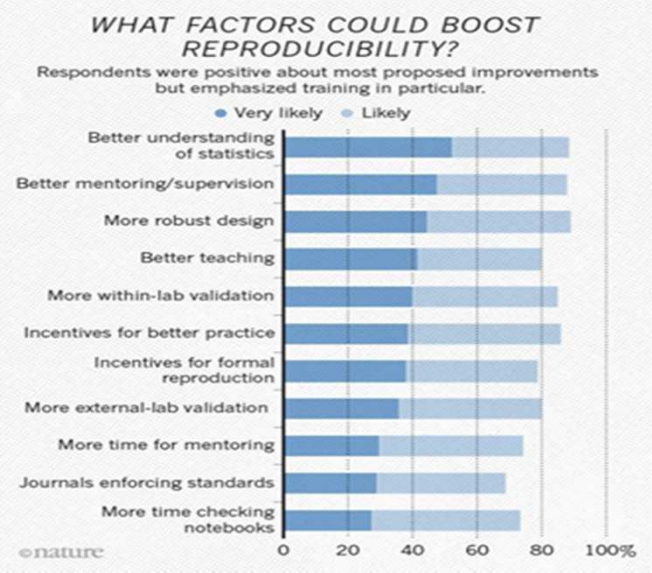
nature International weekly journal of science

Is there a reproducibility crisis in science?

More than 70% of researchers have tried and failed to reproduce another scientist's experiments

More than half have failed to reproduce their own experiments

Nature 533, 452–454 (2016)



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GIVIMP

FOR THE DEVELOPMENT AND IMPLEMENTATION OF *IN VITRO* METHODS FOR REGULATORY USE IN HUMAN SAFETY ASSESSMENT

EURL ECVAM
European Union Reference Laboratory for Alternatives to Animal Testing

We brought together many experts from different sectors and scientific fields to contribute (regulatory, academic, industry)


10 Headings of GIVIMP
Defined at the GIVIMP Guidance meeting

ECHA

efsa

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

OECD Expert groups
BETTER POLICIES FOR BETTER LIVES

EU-NETVAL

EUROPEAN NETWORK OF 37 HIGHLY QUALIFIED GOOD LABORATORY PRACTICE FACILITIES (EU-NETVAL)

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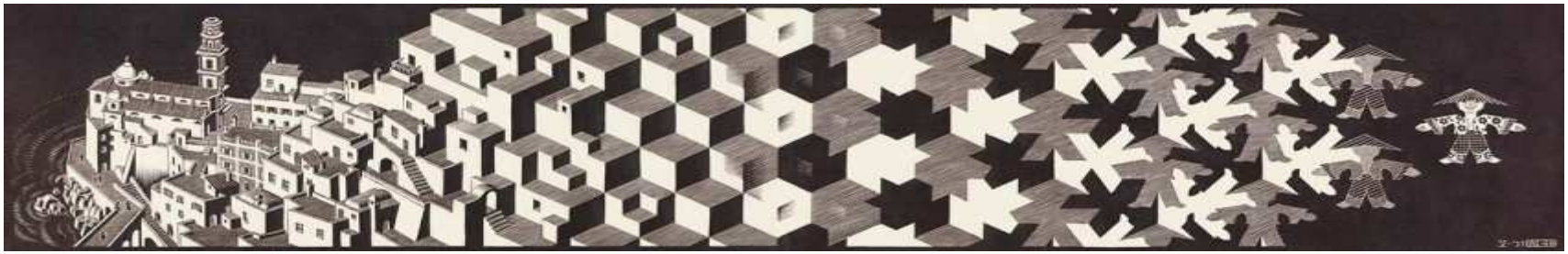
GIVIMP



Metamorphosis by Escher

- ✓ **Well, …nothing this comprehensive exists.**
- ✓ **Bits and pieces exist all over the place but have never been integrated in a holistic way.**

GIVIMP

The GIVIMP GD is divided into 10 sections covering:

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

It combines the current scientific advances with principles of quality assurance and strives for harmonisation at all levels

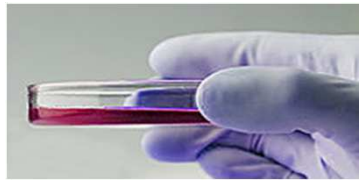
Actors of the Italian Commedia dell'Arte by Jean-Antoine Watteau

The Glider by Leonardo Da Vinci

1 Roles and responsibilities

This guidance document targets all players involved in the process:

- *In vitro* method developers
- Test system (cells, tissues) providers
- Validation bodies
- Producers of equipment, materials and reagents
- *In vitro* method end-users (e.g. EU-NETVAL, testing laboratories, large industries and small to medium enterprises)
- Receiving authorities (EMA, ECHA, EFSA, EPA, FDA, MHLW, MAFF)
- Monitoring authorities (GLP)



2 Quality considerations

Discusses quality assurance versus quality control, quality risk assessment and details quality control requirements for development and implementation of in vitro methods, **the types of documentation needed** and quality considerations regarding the integrity of the data.

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Responsibilities

1

Test system providers

In vitro test systems are mainly biological systems, quite often consisting of tissues or cell lines.

Test systems can be developed in-house (i.e. by the *in vitro* method developer), acquired from other laboratories or purchased from a cell culture bank, either academic or commercial.

The responsibility for the quality and documentation of the test system rests entirely with the test facility, however, the role of the supplier is crucial in aiding the facility meet these quality requirements, e.g. test systems characterisation requirements can often be directly fulfilled by information from the supplier.

Responsibilities

1

Test system providers

It is difficult, if not impossible, to identify cell lines from different origins and ensure that they are not cross-contaminated, misidentified or mixed-up, based solely on morphology and/or culture characteristics.

Authentication techniques are now routinely used both for human and non-human cell lines (5)

Infection or contamination of a cell line with an adventitious virus or mycoplasma may significantly change the characteristics of the cells but again such contamination may not be visibly evident.

1 Responsibilities

Test system providers

The test system provider should therefore provide documentation the cell line's authenticity including verification of its identity and proof to be free of cross-contamination by other cell lines and/or contamination caused by bacteria, yeast or fungi, mycoplasma.

Additional information on the origin and culture history of the cell line, ideally including its transfer among laboratories and repositories, its manipulation (physicochemical or genetic), and details on the types of tests carried out for the detection and (if applicable) elimination of contamination should be made available, so as to provide complete tracking of the cell line provenance.

Responsibilities

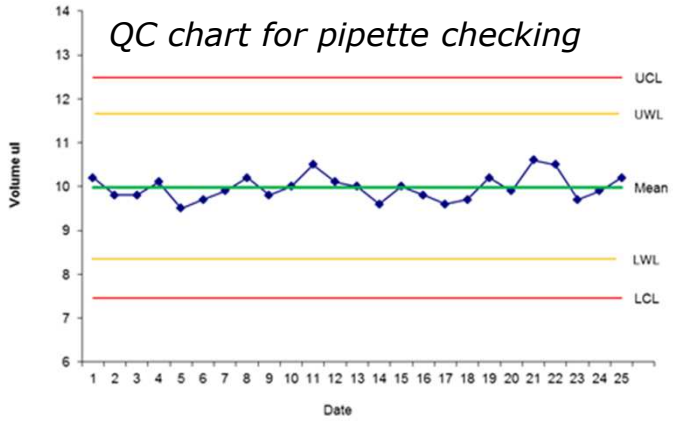
1

Test system providers

In some cases, e.g., cell lines established many years ago may lack some aspects of their provenance and their origin may be unknown. It is therefore recommended to confirm that the cells in current use are assessed against a previously authenticated stock (where available), either in a cell bank or in the laboratory of the originator.

2 Quality considerations

QC chart for pipette checking



Applicability of integrity checks on cell cultures

Attributes	Original Source	Early Stocks	Cell Banks	Routine Cultures
Morphology	✓	✓	✓	✓
Viability	✓	✓	✓	✓[a]
Identity	✓	✓	✓	
Doubling time[b]	✓	✓	✓	✓
Mycoplasma	✓	✓	✓	✓
Viruses	✓ (donor only)		✓ (master bank only)	
Bacteria and Fungi			✓	✓[c]
Function/phenotype		✓	✓	✓[d]
Genetic stability			✓	✓[e]
Absence of reprogramming vectors (iPSC[f] lines)		✓	✓	

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3 Facilities

Proper facility design and management to ensure integrity of test systems and *in vitro* methods and to ensure the production of good scientific and quality results in a safe and efficient manner.

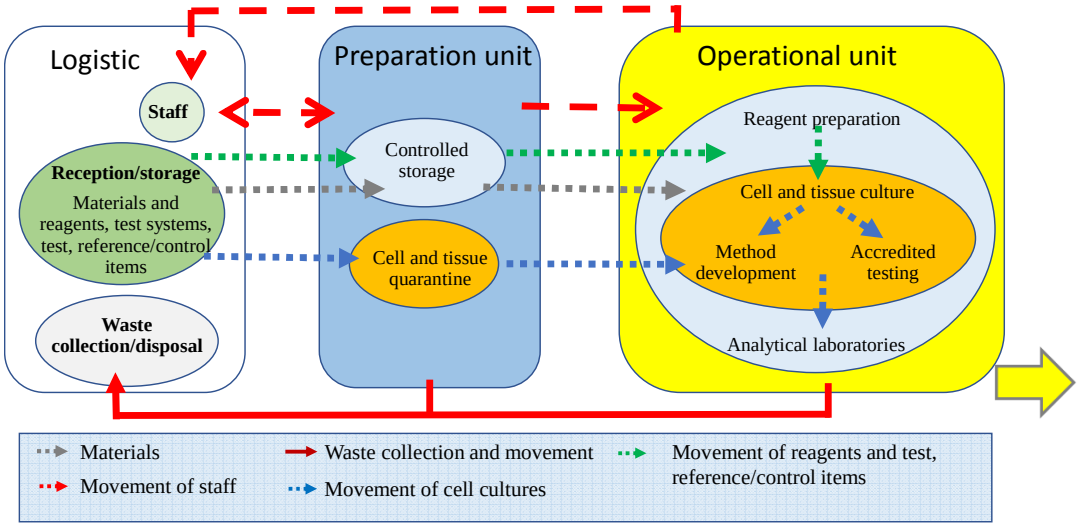
- Safety, risk assessment and management
  (risk groups & biosafety level & biological safety cabinets)
- Facility design
- Containment
- Level of separation to avoid cross-contamination
- Air handling, water supply, environmental control, heating and cooling
- Quarantine for new test systems

4 Apparatus, material and reagents

- Apparatus requirements (including examples of currently available more advanced instrumentation).
- Requirements for material and reagents. (e.g. use of serum, alternatives to use of animal sourced serum, antibiotics, basal & special media, certificate of analysis, stability and traceability).

Facilities

3



Classification	Biosafety Level	Protection Provided	Application
Class I	1, 2, 3	Personnel and Environmental Protection Only	low to moderate risk biological agents
Class II	1, 2, 3	Product, Personnel and Environmental Protection	low to moderate risk biological agents
Class III	4	Total Containment Cabinets	high risk biological agents

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Facilities

3

Classification of laminar flow biological safety cabinet

Classification	Biosafety Level	Protection Provided	Application
Class I	1, 2, 3	Personnel and Environmental Protection Only	low to moderate risk biological agents
Class II	1, 2, 3	Product, Personnel and Environmental Protection	low to moderate risk biological agents
Class III	4	Total Containment Cabinets	high risk biological agents

4 Apparatus, material and reagents

Routine cell and tissue culture according to GCCP should not require the use of antibiotics as it can never be relied on as a substitute for effective aseptic techniques. However, its use is still widespread e.g., OECD TG 432 due to established routine procedures in many laboratories.

Antibiotics are agents that may arrest or disrupt fundamental aspects of cell biology, and, while they are effective against prokaryotic cells (i.e. bacteria), they are also capable of causing toxic effects in animal cells.

Apparatus, material and reagents

4

The use of serum has been discouraged in recent years due to the undefined nature of the medium, batch variability that may contribute to experimental variability and lack of reproducible data, and potential limitation in consistency and availability of supply.

Moreover, in vitro methods, including components, are often developed for legislative or ethical reasons to replace animal methods.

https://fcs-free.org/

Consensus Report

Fetal Bovine Serum (FBS):
Past – Present – Future

Jan van der Valk[1], Karen Bieback[2], Christiane Buta[3], Brett Cochrane[4], Wilhelm G. Dirks[5], Jianan Fu[6], James J. Hickman[7], Christiane Hohensee[8], Roman Kolar[9], Manfred Liebsch[10], Francesca Pistollato[11], Markus Schulz[12], Daniel Thieme[13], Tilo Weber[9], Joachim Wiest[14], Stefan Winkler[15] and Gerhard Gstraunthaler[16]

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Test systems

5

Good Cell Culture Practice – logistics related cell and tissue sourcing, cryostorage, handling, maintenance, identification, containment, authentication and characterisation of the test system (e.g. cell lines, stem cells, primary cells, engineered tissues, etc.)
Already at the development stage.

Test and reference/control items

6

- Test item characterisation, solubility and handling.
- Test item interferences with test system and *in vitro* method.
- Biokinetics, method design considerations during development to ensure test item compatibility and correct and reliable exposure.
- Definition of reference, control items and proficiency chemicals for *in vitro* methods.

Test systems

5

ATLA 33, 261–287, 2005

REFERENCE 02 from EURL ECVAM

Guidance on Good Cell Culture Practice

A Report of the Second ECVAM Task Force on Good Cell Culture Practice

Sandra Coecke,[1] Michael Balls,[2] Gerard Bowe,[1] John Davis,[3] Gerhard Gstraunthaler,[4] Thomas Hartung,[1] Robert Hay,[5] Otto-Wilhelm Merten,[6] Anna Price,[1] Leonard Schechtman,[7] Glyn Stacey[8] and William Stokes[9]

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Test systems

5

Mycoplasma detection methods, their sensitivity, advantages and disadvantages

Method	Sensitivity	Advantages	Disadvantages
Direct DNA stain (e.g., Hoechst 33258)	Low	Rapid, cheap	Can be difficult to interpret
Indirect DNA stain (e.g., Hoechst 33258) with indicator cells (e.g., 3T3)	High	Easy to interpret because contamination amplified	Indirect and thus more time-consuming
Broth and agar culture	High	Sensitive	Slow and may require expert interpretation
PCR	High	Rapid	Requires optimization
Nested PCR	High	Rapid	More sensitive than direct PCR, but more likely to give false positives
ELISA	Moderate	Rapid	Limited range of species detected
Autoradiography	Moderate	Rapid	Can be difficult to interpret if contaminationis at low level
Immunostaining	Moderate	Rapid	Can be difficult to interpret if contaminationis at low level

Test systems

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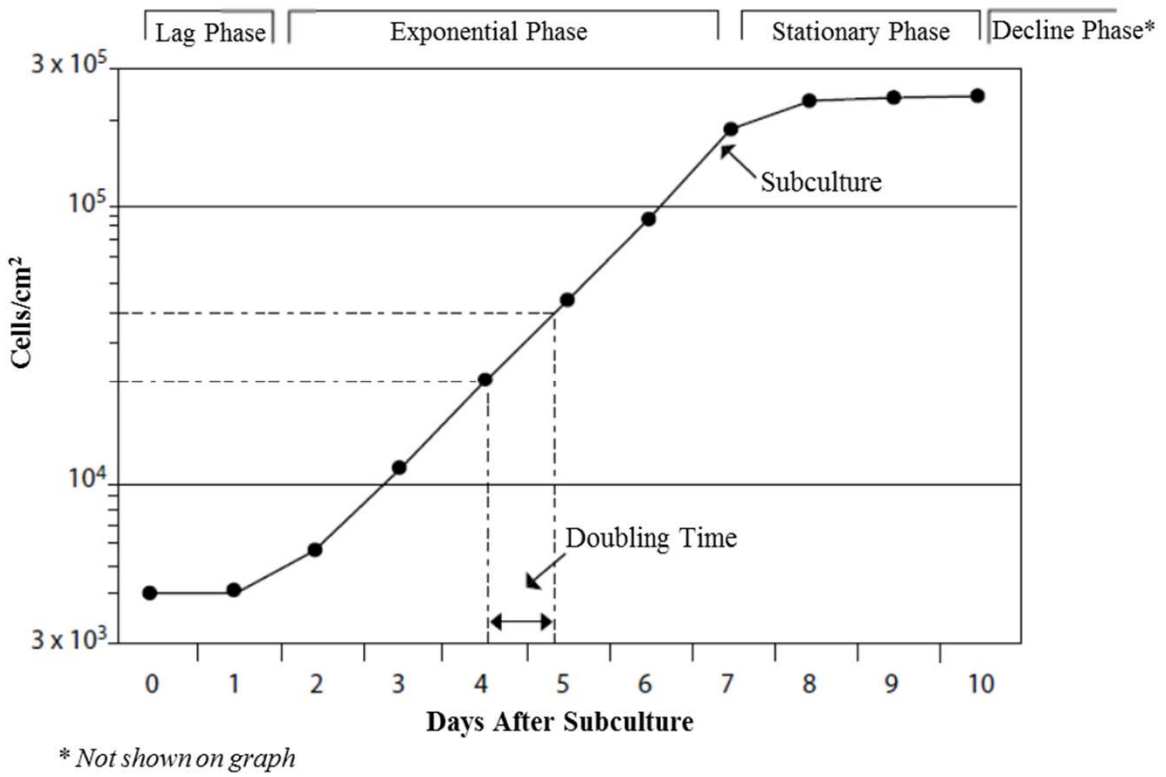
Growth curve for cells grown in culture



* *Not shown on graph*

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Test systems

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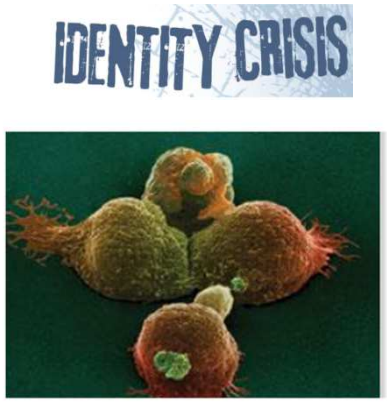

Nature 465, 537 (2010)

Table 1: Cell culture collections (banks)

Cell culture collections	Country	Web site
American Type Culture Collection (ATCC)	USA	http://www.atcc.org
CellBank Australia	Australia	www.cellbankaustralia.com
Coriell Cell Repository	USA	http://locus.umdnj.edu/ccr
Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)	Germany	http://www.dsmz.de
European Collection of Animal Cell Cultures (ECACC)	UK	http://www.camr.org.uk
Japanese Collection of Research Bioresources (JCRB)	Japan	http://cellbank.nihs.go.jp
RIKEN Gene Bank	Japan	http://www.rtc.riken.go.jp
UK Stem Cell Bank (UKSCB)	UK	http://www.nibsc.org/ukstemcellbank

8012–8017 | PNAS | July 3, 2001 | vol. 98 | no. 14 www.pnas.org/cgi/doi/10.1073/pnas.121616198

Short tandem repeat profiling provides an international reference standard for human cell lines

John R. Masters[a,b], Jim A. Thomson[c], Bernadette Daly-Burns[a], Yvonne A. Reid[d], Wilhelm G. Dirks[e], Phil Packer[f], Lorraine H. Toji[g], Tadao Ohno[h], Hideyuki Tanabe[i], Colin F. Arlett[j], Lloyd R. Kelland[k], Maureen Harrison[l], Arvind Virmani[m], Timothy H. Ward[n], Karen L. Ayres[o], and Paul G. Debenham[c]

[a]Institute of Urology, University College London, 3rd Floor Research Laboratories, 67 Riding House Street, London W1W 7EY, United Kingdom; [c]LGC, Queens Road, Teddington, Middlesex TW11 0LY, United Kingdom; [d]American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209; [e]DSMZ German Collection of Cell Cultures, Mascheroder Weg 1b, 38124 Braunschweig, Germany; [f]European Collection of Animal Cell Cultures, Centre for Applied Microbiology and Research, Salisbury, Wiltshire SP4 0JG, United Kingdom; [g]Coriell Institute for Medical Research, 401 Haddon Avenue, Camden, NJ 08103; [h]The Institute of Physical and Chemical Research (Japan) (RIKEN) Cell Bank, Koyadai 3-1-1, Tsukuba Science City, 305-0074, Japan; [i]Japanese Collection of Research Bioresources Cell Bank, National Institute of Health Sciences, 1-18-1 Kami-Yoga, Setagaya-ku, Tokyo 158-8501, Japan; [j]MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9RR, United Kingdom; [k]CRC Center for Cancer Therapeutics, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, United Kingdom; [l]Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, United Kingdom; [m]Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, 6000 Harry Hines Boulevard, Dallas, TX 75235-8593; [n]Department of Drug Development, Paterson Institute for Cancer Research, Wilmslow Road, Manchester M20 4BX, United Kingdom; and [o]Department of Applied Statistics, University of Reading, P.O. Box 240, Earley Gate, Reading RG6 6FN, United Kingdom

Edited by Stanley M. Gartler, University of Washington, Seattle, WA, and approved April 16, 2001 (received for review December 22, 2000)

Test systems

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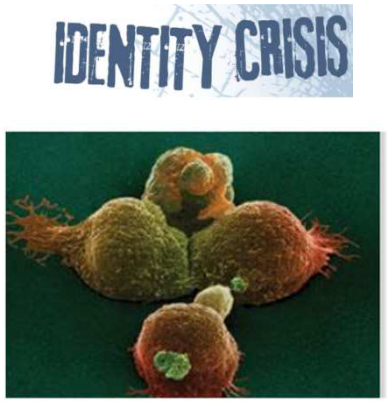

Nature 465, 537 (2010)

Current status of SNP, STR, and DNA barcode technologies as standard methods for assessing the identity of cell lines from different species (Almeida *et al.*, 2016)

Species	Assays	Consensus Standard Method	Commercially Available Kit	Commercial Service	Comparative Data
Human	STR	ASN-0002	Yes	Yes	ATCC, DSMZ, JCRB, NCBI**
	SNP	No	Yes	Yes	(Liang-Chu *et al.*, 2015), (Yu *et al.*, 2015), NCBI
Mouse	STR*	No	No	Yes	Unpublished
	SNP	No	Yes	Yes	(Didion *et al.*, 2014)
African green monkey	STR*	No	No	No	None
Chinese hamster ovary	STR*	No	No	No	None
Rat	STR*	No	No	No	None
Species-level identification	CO1 DNA barcode	ASN-0003	Yes	Yes	Barcode of Life Data System, NCBI**
	Species-specific primers	No	No	Yes	None needed

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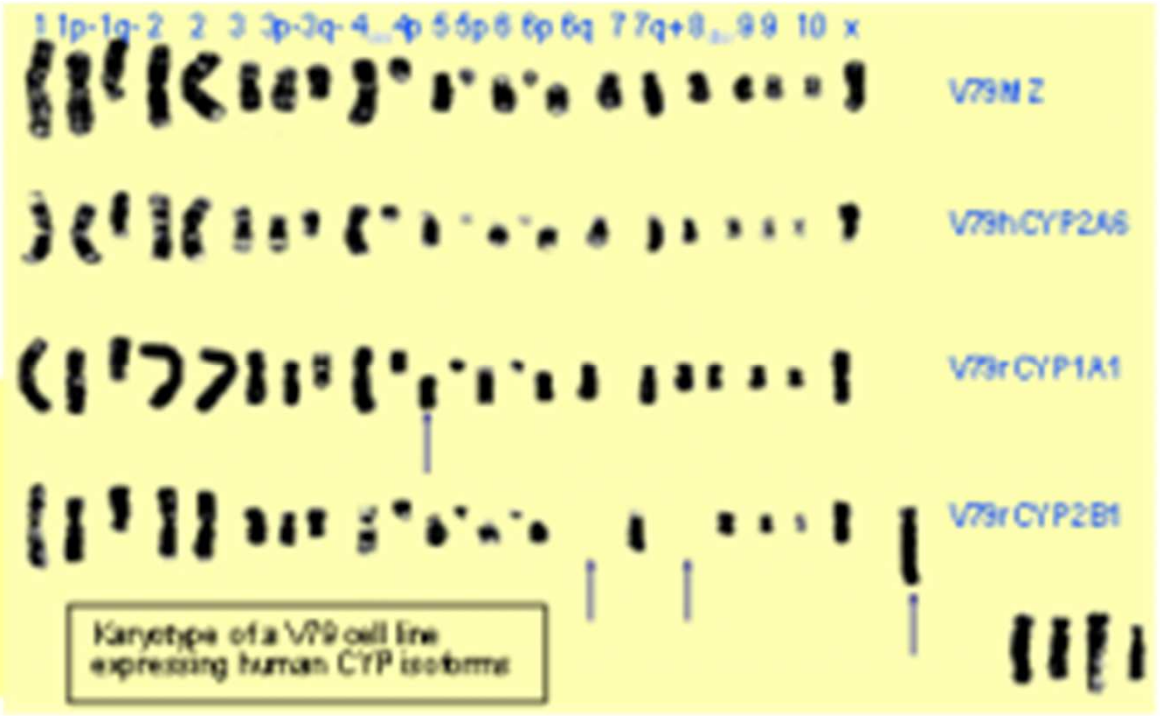
Test systems

5

Genetically modified cell lines



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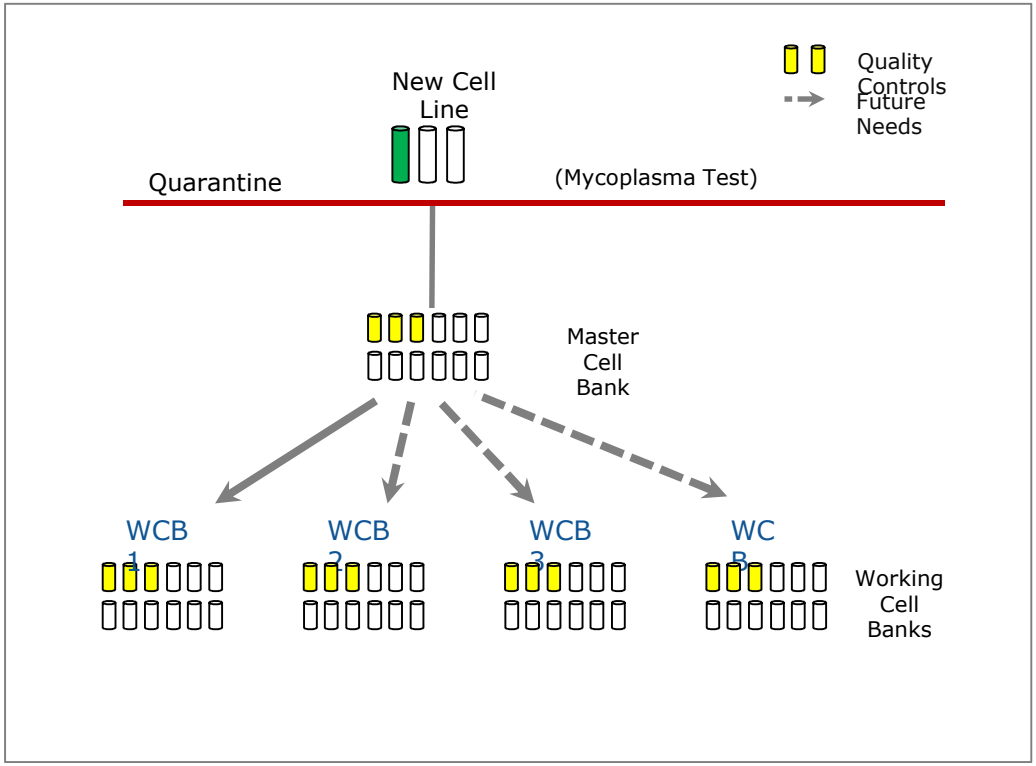
Test systems

5

Cell banking



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Test and reference/control items

6

Common factors affecting solubility

Factor	Affect
Temperature	In most cases solubility increases with temperature, with the exception of gases.
Polarity	In most cases, similar polarities of the solute and solvent increases solubility, e.g., polar solutes do not dissolve in non-polar solvents
Molecular size	As a general rule, excluding other factors, larger particles are generally less soluble
Agitation	Increases the speed of dissolving, i.e. dissolution
Ultrasonification	Increases the speed of dissolving, i.e. dissolution
pH	May affect the solubility of the solute
Pressure	Only affects the solubility of gases

Test and reference/control items

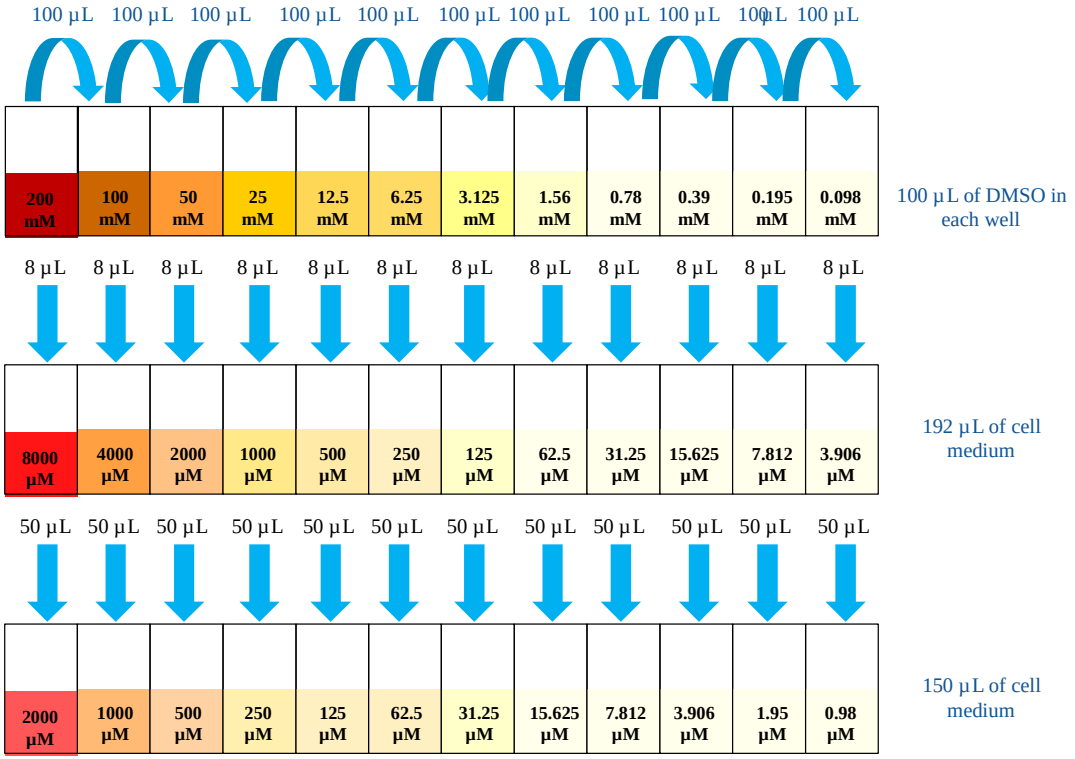
6

Comparison between solubility determination methods

Method	Limitations	Specificity	Cut off	Rapidity
Nephelometry (Light scatter)	• Sticky precipitates • Impurities	Low	No	High
UV/VIS 1 (Absorbance)	• Compound must have chromophore • Sticky precipitates • Impurities	Low	<500 nm	High
UV/VIS 1* (Filtration + Absorbance)	• Compound must have chromophore • Sticky precipitates • Impurities • Loss due to filter absorption	Medium	<250 nm	Medium
HPLC-UV*^	• Sticky precipitates	High	No	Low
LC-MS*^	• Sticky precipitates	High	No	Low
* *Requires Calibration* ^ *High Cost*				

Test and reference/control items

6



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Test and reference/control items

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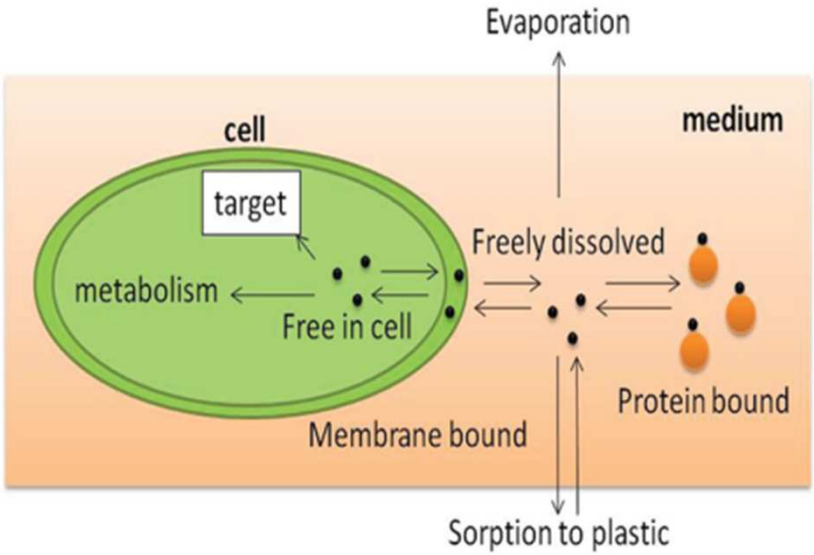

Representation of some processes that can cause the final target concentration to be different than the nominal concentration in an in vitro test (Kramer et al., 2012)

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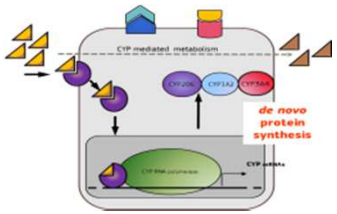
Standard operating procedures (SOPs)

7

Correct setup of *in vitro* methods for routine testing including requirements for clear and concise SOPs.



Performance of the method

8

In vitro method design in the developmental stage: statistical methods for design of experiment; plate layout; data analysis; data-intensive *in vitro* methods; acceptance criteria; dynamic range/range of application; signal intensity; signal variability and plate uniformity assessment; reliability of endpoint calculations; accuracy, reliability and uncertainty.

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Standard Operating Procedures

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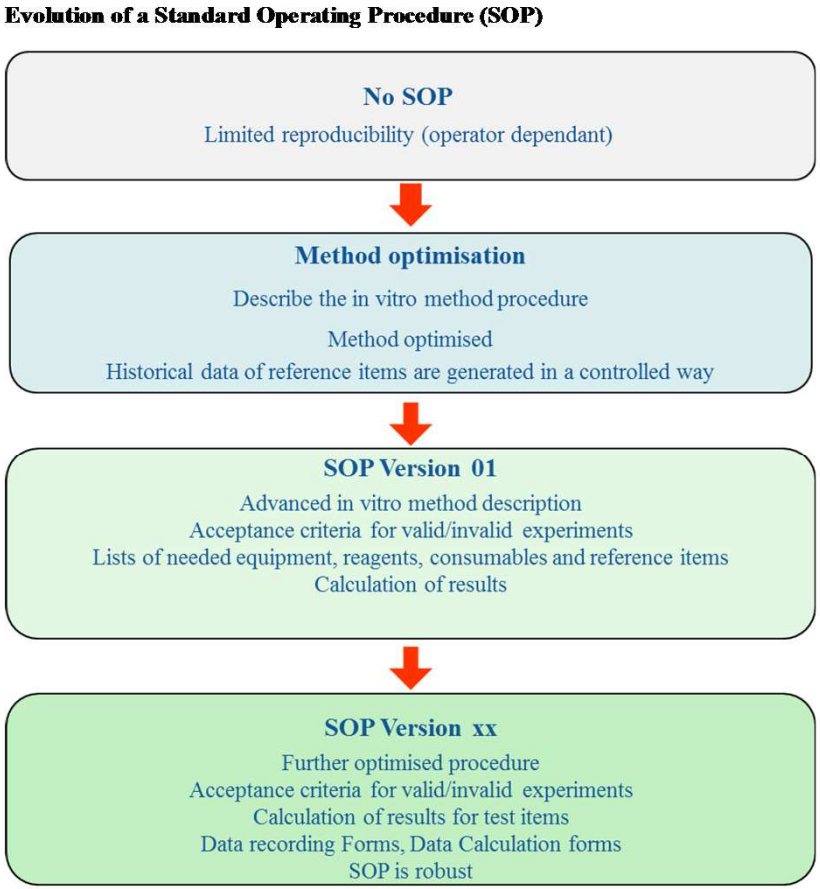

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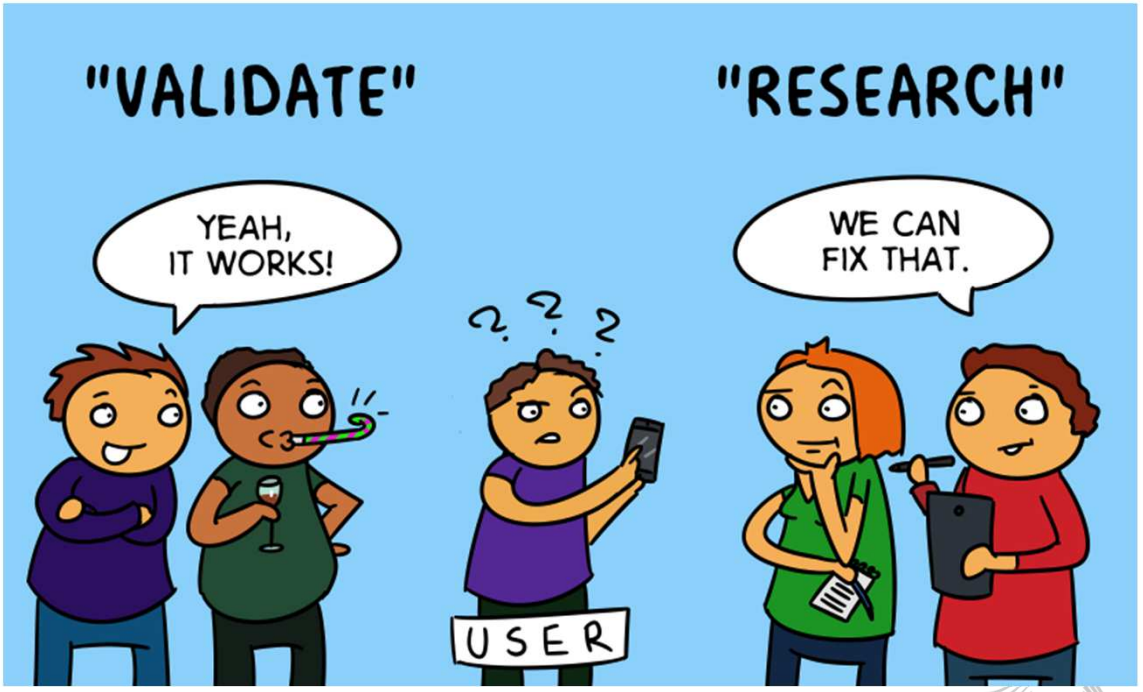
8 In-house validation: a practical example



Performance of the method

8.1 Acceptance criteria

8.2 Experimental design

- 8.2.1 Plate layout
- 8.2.2 Data analysis
- 8.2.3 Outlier detection and removal
- 8.2.4 Non-monotonic dose and U-shaped curves

8.3 In-house validation of the measurement endpoint(s)

- 8.3.1 Detection Limits and Cut-off values
- 8.3.2 Linearity and dynamic range
- 8.3.3 Accuracy and precision
- 8.3.4 Sensitivity and specificity
- 8.3.5 Repeatability

8.4 Proficiency chemicals

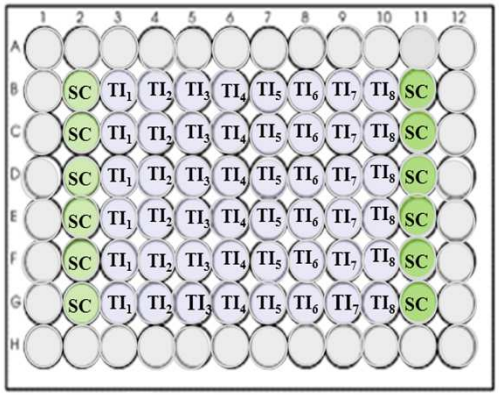
8.5 Data-intensive *in vitro* methods

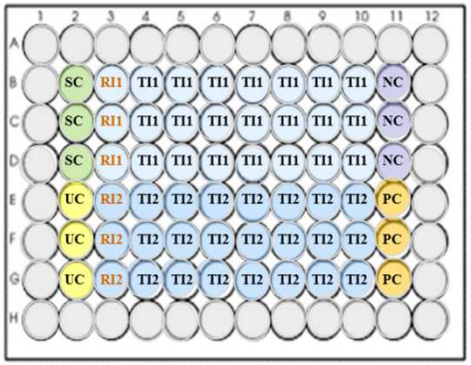
Acceptance criteria

- ✓ Acceptance criteria should primarily be established based on information from historical data.
  - ✓ Historical data should be collected using the unchanged method, unless it can be shown that any changes have not affected the values.
  - ✓ Data should only be rejected when there is a clear, valid and scientifically justified reason to do so, and the reasons for rejecting said data should be clearly and accurately documented.
  - ✓ Can be then supplemented by data from validation studies, or from relevant bibliographic data including guidance documents.
  - ✓ These criteria should be developed and detailed in the *in vitro* method SOP(s).
- ✓ Criteria should be defined for the test system (e.g., passage number, growth curve, cell recovery) and test system performance (e.g., positive, negative, and vehicle controls where applicable).
- ✓ Acceptance criteria should be set for the analytical endpoint determination (e.g., linearity, accuracy, range) and also include data analysis (e.g., line fitting).

Experimental Design

Experimental design is a way to carefully plan experiments in advance so that your results are both objective and valid.





A **factorial experimental design** is used to investigate the effect of two or more independent variables on one dependent variable.

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In-house validation of the measurement endpoint(s)

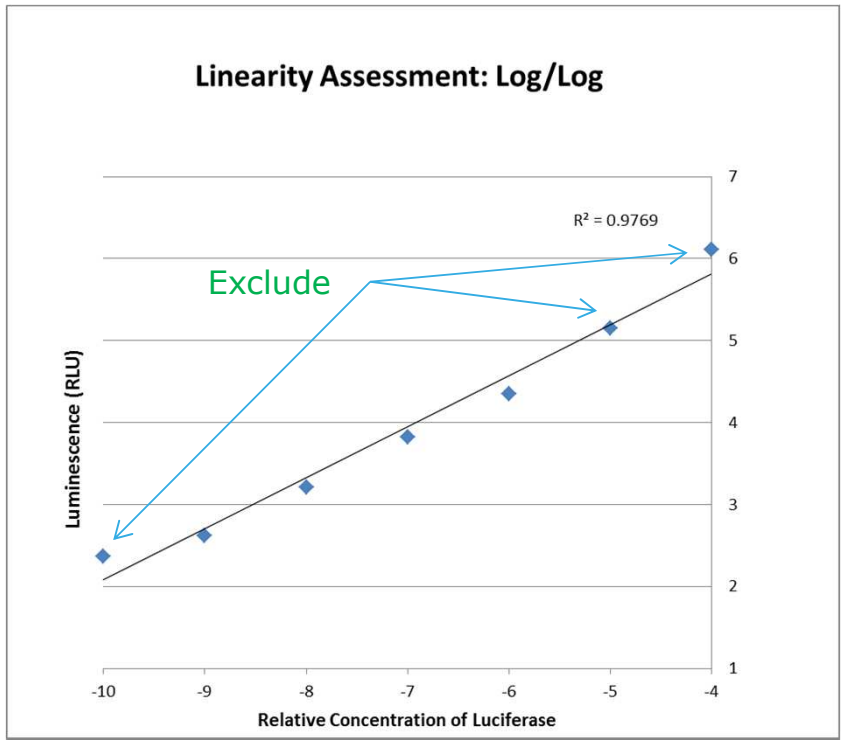
Example: Transactivation assay for the detection of compounds with (anti)androgenic potential using AR-CALUX® cells

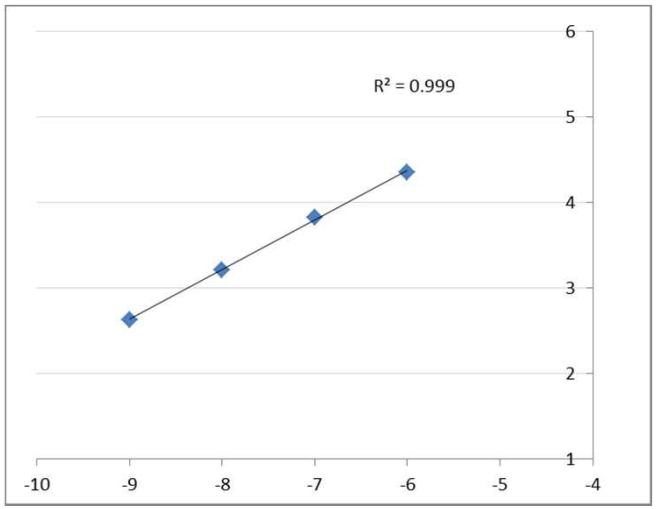
- Determine the linear range in relative light units (RLU). Performed with recombinant luciferase (14.37 mg/ml)

Relative luciferase concentration
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}

Prepare a calibration curve using the recombinant luciferase.

Linearity





- Acceptable linear range 10^{-9} to 10^{-6} relative luciferase concentration corresponds to **4500 to 240,000 RLU**

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Range

Reference item standard curve
1×10^{-4} M
1×10^{-5} M
3×10^{-6} M
1×10^{-6} M
3×10^{-7} M
1×10^{-7} M
3×10^{-8} M
1×10^{-8} M

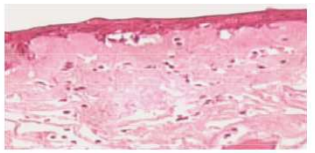
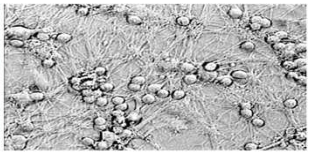
Determined Linear Range: 4500 to 240,000 RLU

Reference item DHT for the agonist AR-CALUX bioassay - range defined in the SOP

Reporting of results

9

Guidance on adequate publishing and reporting of *in vitro* methods, studies and results, including exceptions and deviations.
Data reporting for regulatory purposes.

Storage and retention of records and materials

10

Data integrity, retention and archiving of key records and materials (retrieval, back-up and restore).
Adequate document and record management of processes and the traceability of origin of materials and key decisions.

European Commission

GIVIMP

Is a response to a more general "crisis in reproducibility" in scientific data generation

Accepted by all 35 OECD member countries on the 25th of April 2018 at 18.45.
Now it is send to the Joint Meeting for formal declassification and publication (July 2018)

Draft Guidance Document on Good In Vitro Method Practices (GIVIMP) for the Development and Implementation of In Vitro Methods for Regulatory Use in Human Safety Assessment

(28 FEBRUARY 2018)

Collaboration = faster progress

European Commission

Stay in touch

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