Critical aspects in implementing the OECD monograph No. 14 "The application of the principles of GLP to *in vitro* studies"

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Summary. The Organisation for Economic Cooperation and Development (OECD) principles of good laboratory practice (GLP) were originally developed for application to animal-based toxicology studies. On the other hand, more and more studies involving *in vitro* test systems are performed to produce data on the safety of chemicals with respect to human health and the environment. Therefore, national legislation usually requires that the *in vitro* studies are conducted in compliance with the GLP principles. Furthermore, developments in the area of toxicogenomics, toxicoproteomics, toxicometabonomics and various high throughput screening techniques will also enhance the importance of *in vitro* methodologies for safety testing. The OECD principles of GLP require that safety studies, independent of their type, are planned, conducted, recorded, reported, and archived in a way that they can be inspected by the GLP monitoring authorities and scientifically evaluated by the receiving authorities. Some critical aspects and pitfalls are discussed as regards the proper application and interpretation of the GLP principles for the organisation and management of *in vitro* studies. Organisational charts and responsibilities of test facilities (TFs) involved in single or multisite studies are sometimes dysfunctioning because there is a lack of traceability in reporting and communication lines. Manipulation of cell and tissue cultures of different test systems should be separated and performed under aseptic conditions to prevent cross-contamination. Characterization and environmental conditions under which the test systems are manipulated and stored are critical in *in vitro* studies. Another important pitfall is the lack of description in the experimental design concerning the use of any internal control items to control bias and to evaluate the performance of the test system. Finally, it is observed that samples of long-term preservable test systems are not always retained or only for a short time which can lead to a lack of confirmation of test system identity and/or reconstructability of the study.

Key words: good laboratory practice, *in vitro* studies, *in vivo* studies, critical aspects.

Riassunto *(Aspetti critici nell'adozione della monografia OCSE No. 14 "L'applicazione dei principi di BPL agli studi* in vitro*")*. I principi di buona pratica di laboratorio (BPL) dell' Organizzazione per la Cooperazione e lo Sviluppo Economico (OCSE) sono stati sviluppati inizialmente per essere applicati nell'ambito di studi di tossicologia ambientale. D'altra parte, un numero sempre maggiore di studi intesi ad ottenere dati sulla sicurezza delle sostanze chimiche sotto il profilo della salute e dell'ambiente si basa sull'impiego di sistemi di saggio *in vitro*. Di conseguenza, le normative nazionali richiedono di norma che gli studi *in vitro* siano eseguiti nel rispetto dei principi di BPL. Inoltre, l'importanza delle metodologie *in vitro* per gli studi di sicurezza verrà ancora accresciuta dagli sviluppi nel campo della tossicogenomica, della tossicoproteomica, della tossicometabonomica e delle varie tecniche di selezione ad alta capacità di esecuzione. I principi di BPL richiedono che gli studi di sicurezza, indipendentemente dalla loro natura, siano pianificati, condotti, registrati, riportati ed archiviati in modo tale da poter essere verificati dalle autorità di monitoraggio della BPL e giudicati da un punto di vista scientifico dalle autorità riceventi. Vengono esaminati alcuni aspetti critici e possibilità d'errore nell'impiego appropriato e nella interpretazione dei principi di BPL nella organizzazione e nella gestione degli studi *in vitro*. La documentazione relativa alla organizzazione ed alle responsabilità dei centri di saggio (CdS) impegnati in studi multisito e non è talvolta non idonea a causa della mancanza di tracciabilità nelle modalità di comunicazione e di preparazione dei rapporti. La manipolazione di colture cellulari e di tessuti appartenenti a diversi sistemi di saggio dovrebbe essere condotta in maniera tale da assicurarne la separazione ed aver luogo sotto condizioni asettiche per impedire fenomeni di contaminazione incrociata. La caratterizzazione e le condizioni ambientali sotto cui i sistemi di saggio sono manipolati e custoditi costituisco un aspetto critico negli studi *in vitro*. Un'altra seria mancanza è l'assenza di descrizione nel disegno sperimentale di qualunque controllo interno per accertare deviazioni sistematiche ed il comportamento del sistema di saggio. Infine, è stato osservato che campioni di sistemi di saggio conservabili a lungo termine non sempre sono disponibili o lo sono solo per un tempo breve, con la conseguente probabilità di non poterne confermare l'identità e/o di poter ricostruire gli studi.

Parole chiave: buona pratica di laboratorio, studi *in vitro*, studi *in vivo*, aspetti critici.

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INTRODUCTION

The Organisation for Economic Co-operation and Development (OECD) developed the principles of good laboratory practice (GLP) to promote the quality and validity of test data used for determining the safety of chemicals and chemical products [1]. They have been elaborated by an Expert Group on GLP in 1978 and offer the opportunity to the test facilities (TF) to apply a GLP quality system covering the organisational process and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported. Since 1981 many Consensus and Advisory OECD Documents have been drafted to elucidate some criteria of the OECD principles of GLP. One of these documents is the Advisory Document *"The application of the principles of GLP to in vitro studies"* [2]. The criteria in the OECD Advisory Document have been explicitly interpreted and worked out in a series of workshops organised by the European Centre for the Validation of Alternative Methods (ECVAM) and the International Conference of Variational Methods (ICVAM) [3]. The main goal of the ECVAM evaluation was to promote the scientific and regulatory acceptance of alternative methods which are of importance to the biosciences and which reduce, refine and replace the use of laboratory animals.

The OECD principles of GLP are required to be followed by TFs carrying out studies to be submitted to national authorities for the purposes of assessment of chemicals and other uses relating to the protection of man and the environment. They have been originally written for animal-based toxicological studies. However, there is a growing interest to use *in vitro* methods as an alternative or a supplement to *in vivo* safety testing. The purpose of this combination is to extend the set of toxicological data in order to better evaluate the toxic effects of the chemical substances. *In vitro* methods have been mainly used in the area of genetic toxicity testing where the hazard assessment is based to a large extent on data derived from studies using *in vitro* test systems. There is no doubt that the development of *in vitro* methods in the area of toxicogenomics, toxicoproteomics, toxicometabonomics and various screening techniques will enhance the importance of the *in vitro* methodologies for safety testing. Well known *in vitro* tests are the Ames test (bacterial mutation test), the mouse lymphoma assay (mammalian cell mutation test) and the chromosome aberration test [4-6]. Nearly all mutagens are positive in *in vitro* genotoxicity tests. However, many substances that are positive *in vitro* for genotoxic effects are weakly active or inactive in animals.

In the whole concept of the OECD principles of GLP four important players are involved: the sponsor, the Regulatory Authority (RA), the monitoring authority, the TFs. Therefore, it is extremely important that the OECD principles of GLP are complemented by criteria that facilitate their proper application and interpretation to the organisation and management of *in vitro* studies and to provide guidance for the appropriate application of the GLP principles to *in vitro* studies to the actors concerned.

The purpose of this article is:

a) to consider some critical aspects concerning the application of the OECD principles of GLP to *in vitro* studies;
b) to provide some clarifications related to the implementation of the OECD principles of GLP to *in vitro* studies;
c) to assist the GLP inspectors in the context of *in vitro* study audits.

SCOPE OF THE OECD PRINCIPLES OF GLP

The OECD principles of GLP should be applied to the non-clinical safety testing of test items contained in pharmaceutical products, pesticide products, cosmetic products, veterinary drugs as well as food additives, feed additives and industrial chemicals. These principles apply to all non-clinical health and environmental safety studies required by regulations for the purpose of registering or licensing pharmaceuticals, pesticides, food and feed additives, cosmetic products, veterinary drug products and similar products, and for the regulation of industrial chemicals. The same scope holds in the case of non-clinical safety *in vitro* studies.

The purpose of testing the above test items is to obtain data on their properties and/or their safety with respect to human health and/or the environment. These test items are frequently synthetic chemicals, but may be of natural or biological origin and, in some circumstances, also living organisms.

The Decision of the OECD Council concerning the mutual acceptance of data (MAD) in the assessment of chemicals [C(81)30(Final)] states that data generated in the testing of chemicals in an OECD member country in accordance with OECD test guidelines (TGs) and OECD principles of good laboratory practice shall be accepted in other member countries for purposes of assessment and other uses relating to the protection of man and the environment. Therefore, it should be required that *in vitro* toxicology studies are validated to be in compliance with the OECD principles of GLP in order to facilitate their acceptance by OECD member countries [7, 8]. However, some national legislation extend this scope to other products (*e.g.* medical devices) or types of testing (*e.g.* bioavailability, clinical trials) which can unbalance the harmonization of the monitoring systems and create trade barriers. Therefore, it is recommended that the sponsor and the TF inform them correctly about the requirements of the national GLP MAs before starting a study. A close relationship between the receiving and the MAs, on the one hand, and continuous and understandable information to the sponsors and TFs, on the other hand, is strongly recommended in a GLP environment *(Figure 1)*.

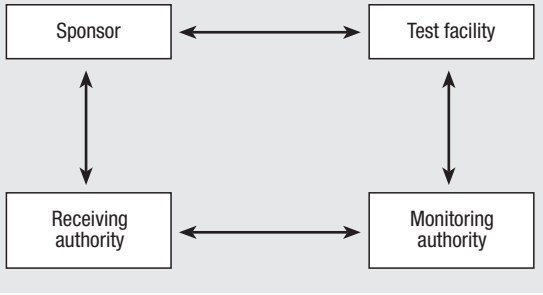

Fig. 1 | *Relationships between the different parties in a GLP environment.*

In the same TF both regulatory studies and non-regulatory studies can be performed. In the former case they are submitted to the RAs for scientific evaluation, but not in the latter case. However, if GLP and non-GLP studies are performed in the same TF it is recommended to carry out both types of studies according to the same GLP quality system, although for the non-GLP studies the QA programme and the archiving are not considered to be critical issues.

RESPONSIBILITIES

The OECD principles of GLP, issued in 1981, have been published for single-site studies. It means that the organization and all the experimental work of the study concerns only one location. The most important roles and responsibilities in such studies are defined for the sponsor, the TF management (TFM), the study director (SD), the QA manager (QAM) and the archivist *(Figure 2)*. To ensure the integrity of the studies it is recommended that the QAM and the archivist are not involved in the study. Therefore, it should be clear in the organization structure of the TF organisation that the QAM and the archivist are indicated as independent units who directly report to TF management.

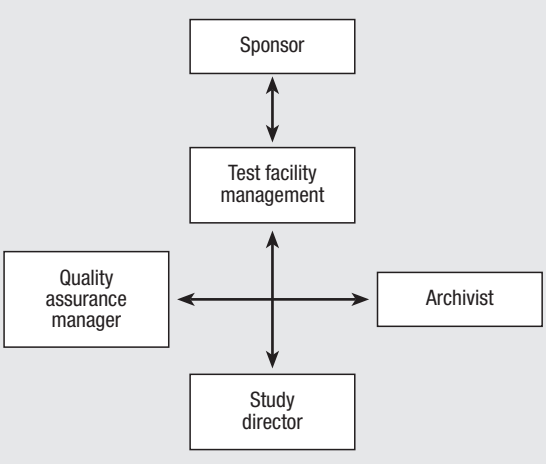

Fig. 2 | *Organisation structure of a single-site study.*

They have to be designated by the TFM and they should report to the TFM. This situation is not clear in many TFs, *e.g.* reports to the TFM are missing and no corrective actions are taken by TFM when serious deviations have been observed.

Since the last decade more and more studies are split in delegated phases carried out at different geographical locations. They have been defined as multi-site studies [9]. The organization and management of such studies can be very complex and clear communication lines are necessary between the key functions to ensure a complete reconstructability of the study *(Figure 3)*. As in the case of a single-site study, a multisite study has only one SD, one study plan and one final study report.

In multisite studies it is very important that the SD communicates with the responsible persons of the test site(s) (TSs) before the study starts to ensure that the delegated phase is performed by a principal Investigator (PI) who is scientifically qualified and trained in the application of the OECD principles of GLP. PIs are obliged to prepare contributory reports on their work for the SD. In several cases these reports are missing and only raw data are sent to the SD. The communication lines with QA officer at the TS are even worse. Some QA reports are not available and it is not always clear to whom the QA reports have been sent.

To audit multisite studies it is very important to verify the organizational charts of the TSs involved to detect the responsibilities at the different levels. In some cases it could be necessary to carry out joint inspections with other GLP MAs to obtain a clear picture of the whole study. According to the OECD principles of GLP the TFM can consist of an individual or a team of individuals who should ensure that the said principles are complied with. They are of general nature and can equally be applied to *in vivo* and *in vitro* studies. In many cases a policy document identifying the TFM is missing and the individual(s) indicated to play the role of TFM cannot prove how they discharge their duties. During the inspections it is sometimes observed that qualified staff is not available and that particular *in vitro* training programmes are missing. It is important that the inspectors ask for a list of SDs and PIs mentioning the date when they have been acknowledged competent for their function and the date when they quitted as SD or PI. This list should be approved and updated by the TFM. This check is very important to ensure that the study and the delegated phases, if applicable, have been conducted by qualified personnel *(Figure 4)*. Another critical aspect of *in vitro* studies is the availability of appropriate areas, equipment and materials to carry out their various phases under the best conditions. Separation of activities and the correct implementation of well detailed procedures are required to ensure that contamination

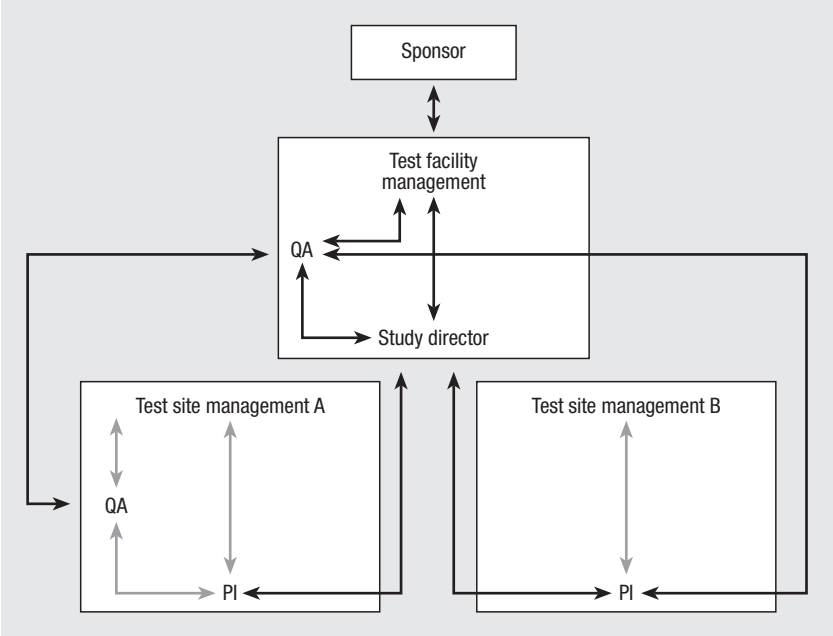

Fig. 3 | *Organisation of a multisite study.*

of test systems is avoided. The materials (*e.g.* kits) delivered by the suppliers should meet the requirements written down in the experimental design of the study plan. It is recommended to ask for certificates specifying the characteristics of the materials. Even if the supplier guarantees the quality of materials and products it is still necessary that the TFM schedules inspections to verify that the suppliers fulfil their specifications and that their practices, procedures and policy are assessed.

In the case of multisite studies where the *in vitro* experiments are delegated phases, communication lines between the SD, PI(s), QA programme(s) and study personnel should be defined before the study starts. The same applies to responsibilities concerning the performance of the experiments, the documents to be followed and the type of reports to deliver. This can be done in the minutes of a meeting or in the study plan. It is recommended that these responsibilities are approved by TFM and TS management(s) [TSM(s)].

The responsibilities of the SD as defined in the OECD principles of GLP also apply to *in vitro* studies. The SD functions as the single point of study control in single-site studies as well as in multisite studies. It is extremely important that he/she pays attention to the justification and characterization of the test system and document it. The test method used should be validated or should be proven to be structurally, functionally and/or mechanistically similar to a validated reference test method. It means that comparable performance is obtained

Fig. 4 | *Qualified technician evaluating cell culture ©EC (2007).*

when evaluated with appropriate reference items. The second important issue of the test system is its characterization. Correct and suitable information about the origin and type of cell lines, age/number of passages and evaluation with appropriate reference items, including positive, negative, untreated and/or vehicle controls, if necessary, should be traceable. In this context, the inspectors should verify that the SD can ensure that the performance of these materials and kits is comparable with the specifications provided by the supplier, meets the requirements written down in the experimental design of the study and is suitable for the intended purpose. Therefore, the SD should be able to show that the completeness and acceptability of the quality control documentation provided by the supplier has been examined and critically evaluated. The GLP inspector should also verify that the study plan, amendments to the study plan and final report are signed and dated by the SD. In some cases it can be observed that raw data used in the discussion of the results of the study are already obtained before the start of the study. It means that this part of the study has not been controlled by the SD, which is not acceptable. Another critical aspect of SDs' responsibilities is the lack of traceability regarding the follow-up of the study. It cannot be demonstrated that monitoring activities and type and frequency of reviews are documented in the study records.

If *in vitro* experiments are integrated in multisite studies responsibilities of the TSM should be defined. They are the same as those for TFM with the exception of a number of cases, as detailed in *Table 1*.

Although it is not mentioned in the GLP principles, it would be more realistic if the TFM were the only responsible for the characterization of the test item because the TFM and SD are those who negotiate directly with the sponsor as regards the characteristics of the test item.

If *in vitro* tests are carried out in the context of a multisite study the role and responsibilities of the PI should be defined because he/she will ensure that the delegated phases of the study are conducted in accordance with the GLP principles. Hence, the PI is obliged to communicate regularly with the SD and each deviation should be addressed in a timely manner. If a planned change takes place during the experiments an amendment should be issued and approved by the SD before the study can be continued. One of the main problems encountered in the relationship between the SD and the PI is the way of reporting. Some PIs only send raw data to the SD for inclusion in the final report. In other cases, PIs prepares an intermediate report which is attached to the final report. In most cases intermediate reports also contains a QA statement specifying the inspections carried out. Taking into account the different possibilities and responsibilities between the key persons involved in the study it should be defined before the start of the study what should be done by whom, when and how.

Table 1 | *Limited responsibilities of the TSM*

1.1.2.g-h	TSM cannot appoint a SD and his/her replacement, but should designate the principal investigator. Of course, the qualification of the principal investigator should be discussed with the SD and TFM.
1.1.2.i	TSM cannot request the SD to sign the study plan. On the other side, it is recommended that TSM and pincipal investigator sign the study plan to prove that they are agree with the delegated phase of study.
1.1.2.j	TSM cannot impose the SD to make copy of the study plan available to the QA personnel.
1.1.2.o	TSM cannot ensure that clear communication lines exist between the SD, PI and staff personnel.

The responsibilities of study personnel are shown in *Table 2*.

QA PROGRAMME

The requirements for the QA activities are not so different between *in vitro* and *in vivo* studies. However, the GLP inspectors should have a good knowledge of the criteria described in the OECD consensus documents No. 4 and 7 on QA and GLP and the application of the GLP principles to short-term studies [12, 13]. Such type of studies may be inspected by the QA Unit on a processed-based inspection programme, if permitted and applicable by national regulations. If allowed, the QA programme of the TF or TS should define at random the frequency of inspections that will be carried out on such studies. One of the most important aspects of such an inspection programme is the indication of critical phases. They should be defined by consent with the SD, PI and study personnel. Taking into account the specificity of the *in vitro* studies education and training of QA personnel it is necessary to recognize that there may be potential problems in specific

Table 2 | *Study personnel's responsibilities*

1.4.1	All personnel should have a good knowledge of those parts of the GLP principles which are necessary for the performance of the study. They have to follow the requirements of aseptic conditions to avoid any kind of pathogen contamination of the test system
1.4.2	They have to follow strictly the instructions of the study plan and the procedures concerned to avoid cross-contamination between test systems and to ensure the integrity of the study. Any deviation from these instructions should be communicated to the SD and PI(s), if appropriate
1.4.3	Raw data should promptly and accurately be recorded with reference to the individual entering the data.
1.4.4	They should respect the health precautions to be taken to minimize risk to them and to ensure the integrity of the study. In this context, they should be aware and strictly adhere the requirements to isolate the test systems and studies involving bio hazardous chemicals

Table 3 | *Important areas for inspection in* in vitro *testing*

2.2.1.c	Monitoring of batches of components of cell and tissue culture media that are critical to the performance of the test system (*e.g.*; foetal calf serum etc.) and other materials with respect to their influence on test system performance
2.2.1.c	Assessing and ensuring functional and/or morphological status (and integrity) of cells, tissues and other indicator materials
2.2.1.c	Monitoring for potential contamination by foreign cells, mycoplasma and other pathogens, or other adventitious agents, as appropriate
2.2.1.c	Cleaning and decontamination of facilities and equipment and minimizing sources of contamination of test items and test systems
2.2.1.c	Ensuring that specialised equipment is properly used and maintained
2.2.1.c	Ensuring proper cryopreservation and reconstitution of cells and tissues
2.2.1.c	Ensuring proper conditions for retrieval of materials from frozen storage
2.2.1.c	Ensuring sterility of materials and supplies used for cell and tissue cultures
2.2.1.c	Maintaining adequate separation between different studies and test systems

areas of *in vitro* testing. Some important specific areas are summarized in *Table 3*.

FACILITIES

The infrastructure of the facilities should be of adequate and suitable design with required capacity to ensure an adequate degree of separation between the different activities and a proper and undisturbed conduct of each study. Taking into account that the *in vitro* studies generally need limited workplaces appropriate separation is an absolute priority if *in vitro* studies are performed at the same time in the same physical environment. The important aspect here is that the integrity of each test system and the study should not be jeopardized by the possibility of potential contamination, cross-contamination or mix-up.

However, it is possible that cells or tissues belonging to different studies are incubated in the same apparatus under the condition that appropriate identification is visible and that labelling of the different test systems is correctly and legibly carried out. In the case of co-existing testing the SD should be aware of the characteristics of the test items used to avoid that interferences due the volatility of some test items can occur during co-incubation. Mixing of test items or test systems is also a real danger when administration of the test items to the test systems is performed.

In this context, separation of critical phases on a spatial or temporal basis is recommended. Therefore, the GLP inspectors should verify how the manipulation of cell and tissue cultures (*e.g.*; subcultivation procedures, addition of test item etc.) is performed. Mostly vertical laminar flow cabinets are used to assure sterility and to protect the test system as well as the study personnel and the environment. Sequential manipulation of test systems used in individual studies will prevent cross-contamination between different studies under the condition that careful cleaning and decontamination/sterilization of the working surfaces of the cabinet and of related laboratory equipment are carried out by well trained personnel.

For long-term storage of test systems rooms or areas with special equipment should be available. The use of cryo-preservative containers can be necessary and temperature and liquid nitrogen level should be monitored and recorded.

In *in vitro* studies it frequently happens that the test and reference items are mixed with vehicles. This activity should be done in suitable rooms or areas under aseptic conditions to minimize the possibility of contamination of the test system by the test and reference items thus prepared.

APPARATUS, MATERIAL AND REAGENTS

The applicability criteria of the GLP principles for *in vivo* studies coincide with those for *in vitro* studies. However, proper conditions of certain equipment should be documented, monitored and recorded. In *in vitro* studies microbalances, micropipettes, laminar air flow cabinets or incubators should be regularly maintained, monitored and calibrated as necessary. Critical parameters should be identified and monitored to check whether the pre-established limit values are respected and to identify the causes in case they are exceeded. The installation and functioning of alert systems is very important to ensure the integrity of the experimental phase as well as of the whole study. In this context, it is necessary to check the incubator temperature at different places with and without material inside to assure that the differences in temperature in the incubator remain within acceptable limits. An important parameter to check the incubation process is the use of positive, negative and vehicle control samples.

TEST SYSTEMS

As defined in the OECD advisory document No. 14 the *in vitro* studies do not use multicellular whole organisms, but rather microorganisms or material isolated from organisms or simulations thereof as test systems. Even if this kind of test systems are less complex than a whole organism, there are still a lot of parameters which might interact with the test items and perform art factual reactions. It is for this reason that the selection of a given test system for a certain study can be unrealistic. This choice is influenced by predefined criteria such as

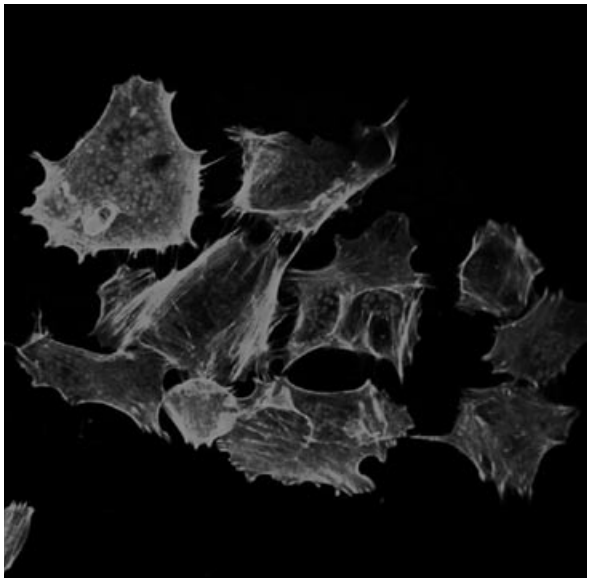

Fig. 5 | *Example of cell cultures.*

viability, suitability and responsiveness of the test items *(Figure 5)*.

For this reason it is necessary to obtain detailed documentation about the test systems, not only as regards the intrinsic characteristics (linked to the identity of the test system), but also about predefined criteria like the viability, suitability and responsiveness of the test system. Relevant information such as the origin, age/passage numbers and cell doubling should be reviewed and additions and modifications should be recorded and retained in the study file. This information has a direct link with the characteristics of the test system and their changes during a period of time. Several publications have stressed the importance of the passage number on the characteristics of a cellular culture. A recent study has shown that a number of 7 to 12 passages could trigger morphological abnormalities in stem cells, attenuate the expression of specific surface markers and ultimately cause proliferation arrest [10].

Once the choice of the test system is justified, it is necessary to develop in the facility adequate conditions to control the integrity and the quality of the test system and to maintain and to monitor these conditions before, during and after their use in the study. Environmental conditions related to the storage of the test systems and the experiments on test systems should be directly and unambiguously monitored, recorded and archived to assure the reconstructability of the study, *e.g.* temperature and CO_2 concentration in the incubator should be observed, monitored and recorded during the study. In the case of cell culture, the facility has to set up a test to check the absence of mycoplasma in the culture. Mycoplasma contamination in cell culture is a serious setback to cell culturists across the world with a very high rate of reported occurrence particularly because of difficult early detection [11]. It is for this reason that the detection test has to be regularly performed and the result of this assay should be retained in the study file.

Manipulation of cell and tissue cultures should be performed under aseptic conditions to prevent contamination *(Figure 6)*. Each manipulation of the test system can be the cause of a contamination which can directly affect and even destroy the test system. The facility has to develop adequate conditions of manipulation to avoid such a problem. As an example, all the materials directly in contact with the cell of tissue cultures have to be sterile or sterilised to avoid the problem of contamination. Records of sterilisation should be kept in the study file. In the same way, laminar air flow cabinets or incubators have to be regularly maintained and monitored. This monitoring should include sterility tests, for instance by the use of the contact plates.

All the manipulations and treatments, *e.g.* with antibiotics or antifungals, selective cultivations subcultivation etc., should be recorded. The reason for this is linked to the influence of such a manipulation on the integrity and quality of the test system. A product like an antibiotic can cause a stress and affect by this way the integrity of the test system. In the same way a treatment of the test system can affect the interaction between the test system and the test items and lead to art factual results.

The GLP principles require also that records of the source and information on the date of arrival and the arrival conditions of test systems be kept.

Special attention should be paid to the labelling of the test systems during the storage and the use. The cryostorage in liquid nitrogen can be critical for this labelling. It is for this reason that a durable labelling should be chosen to ensure the correct identification of the test system at all times. The facility has also to pay special attention for the conservation system and the critical parameters should be recorded (*e.g.* records of the liquid nitrogen level in a liquid nitrogen should be monitored and kept in the study file).

In the case of test kits used as test system, records of their physical-chemical characteristics and unambiguous labelling with the indication of the ex-

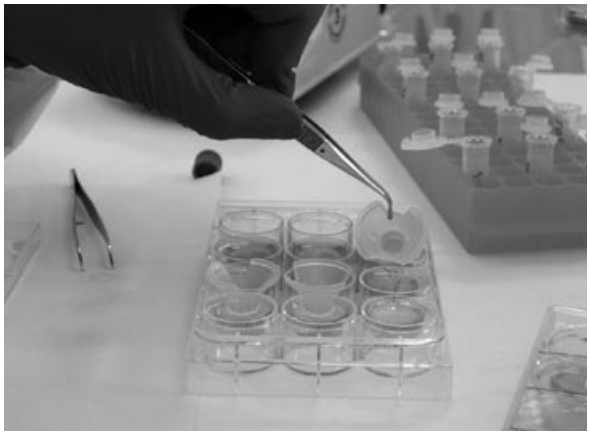

Fig. 6 | *Example of manipulation of cell culture.*

piry dates are requested. Extending this expiry date is only acceptable if evaluation is justified by documental evidence (*e.g.* re-analysis). This evaluation may consists of the historical data of the analysis of the relevant batch of the test kit to positive, negative and/or vehicle control items. The results of re-analysis should prove that they do not significantly differ from historical control values.

TEST, REFERENCE AND CONTROL ITEMS

In vitro studies, control items can be defined as positive, negative and/or vehicle control items. They are not considered as reference items because they serve in monitoring the performance of the test system, but might not be necessarily compared with the test item in the same way. During the inspections it is observed that a lot of test facilities do not understand very well the difference between the three types of items which lead to a wrong description of the items in the study plan.

As for the general GLP principles, records of receipt, handling, sampling, storage and characterisation of the test and reference items are required and should be kept in the study file. It is not always necessary to determine the concentration and the homogeneity of the control items because the test systems can provide sufficient evidence for the correct, expected response to them.

The expiry date of the control items may also be extended by documented evaluation or analysis on the basis of historical control values which can be compared with published reference values.

In the case of cell or tissue cultures, the sterility of the test and reference items could be required. A sterility test has to be regularly performed and the result of this should be retained in the study file. In the same way, the handling of the test/reference items in aseptic conditions is also necessary to avoid microbial contamination of the test systems.

STANDARD OPERATING PROCEDURES

The applicability criteria of the GLP principles quoted in 7.1-7.4 coincide for *in vivo* and *in vitro* studies. It means that the standard operating procedures (SOPs) required by the GLP principles for test and reference items, apparatus, materials and reagents, report keeping, reporting, storage and retrieval of records and materials, test system and quality assurance programme should also be available for *in vitro* studies as well. Nonetheless, some activities and processes are so specific to *in vitro* testing that particular SOPs should be written and available. A few examples are summarized in *Table 4*.

PERFORMANCE OF THE STUDY AND REPORTING OF STUDY RESULTS

The GLP requirements for the performance of *in vitro* studies are identical to those provided for the more conventional safety studies. However, there are a number of issues specific to *in vitro* testing that should be addressed in the experimental design of the study plan as well as in the final study report. One of the important issues that should be taken into account is of scientific nature. The TF should introduce in the study plan the requirement that any internal controls, used to check the bias and to evaluate the performance of the test system, should be conducted concurrently with the test item.

Other specific requirements can be found in the relevant OECD TGs or appropriate references.

Special attention should be paid to the following issues:

- justification for the selection of the test system;
- characterization of an *in vitro* test system(s), such as species and tissue of origin, source of supply, cell designation, culture conditions and other relevant information;

Table 4 | *Illustrative examples of SOP specific for* in vitro *testing*

7.4.a	*Facilities* Environmental monitoring with respect to pathogens in the air and on surface, cleaning and disinfection. Actions should be described in the case of infection or contamination in the test facility or area
7.4.b	*Apparatus* Use, maintenance, performance monitoring, cleaning and decontamination of cell and tissue culture equipment and instruments, such as laminar-flow cabinets and incubators Monitoring of liquid nitrogen levels in storage containers Calibration and monitoring of temperature, humidity and CO2-levels in incubators
7.4.c	*Materials, reagents and solutions* Evaluation of suitability, extension of expiry dates, assessment and maintenance of sterility, screening for common pathogen contaminants Description of procedures for choice and use of vehicles Verification procedures for compatibility of vehicles with the test system
7.4.d	*Test systems* Conditions for storage and procedures for freezing and thawing of cells and tissues Testing for common pathogens Visual inspection for contaminations Verification of procedures for ensuring properties and responsiveness on arrival and during use, whether immediately after arrival or following storage (*e.g.*; acceptance criteria). Morphological evaluation, control of phenotype or karyotype stability, control of transgene stability Mode of culture initiation, culture conditions with subcultivation intervals Handling of biohazardous materials and test systems, procedures for disposal of test systems
7.4.e	*Performance of the study* Aseptic techniques Acceptance criteria for study validity Criteria for assay repetitions
7.4.f	*Quality assurance* Definition of critical phases Inspection frequency

- number of treatment groups and replicate determinations within each treatment group;
- use of positive and negative control items;
- assay acceptance criteria for endpoints;
- a list of records to be retained, including their location.

STORAGE AND RETENTION OF RECORDS AND MATERIALS

The requirements of the GLP principles should also be applied to *in vitro* studies. Except the archiving of records and test and reference items samples of long-term preservable test systems such as special subclones of cell lines, transgenic cells, should be considered to confirm test system identity and/or study reconstructability.

Finally, records of historical positive, negative and untreated and/or vehicle control results used to establish the acceptable response range of the test system should also be retained.

CONCLUSIONS

The *in vitro* studies performed in compliance with the GLP principles promote confidence in experimental data and reporting. The *in vitro* studies can be performed in GLP and non-GLP areas. The TF should ensure that the GLP areas are not influenced by the non-GLP areas.

The *in vitro* studies might be conducted for regulatory and non-regulatory purposes. It is recommended to carry out both types of studies according to the same GLP quality system to avoid deficiencies. The main difference is that the QA programme and the archive requirements can considerably be reduced for non-regulatory *in vitro* studies.

The applicability of GLP to *in vitro* studies should take into account some additional requirements concerning the handling and storage of test items, the characterization and care of test systems, the required use of positive and negative control items and acceptance criteria.

The *in vitro* studies can be considered as screening methods complementary to *in vivo* studies. Nonetheless, they should be thoroughly validated as any other test.

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References

1. Organisation for Economic Co-operation and Development. *OECD principles on good laboratory practice (as revised in 1997)*. Paris: OECD; 1998. (OECD Series on principles of GLP and Compliance Monitoring, No. 1, ENV/MC/CHEM(98)17). Available from: http://www.olis.oecd.org/olis/1998doc.nsf/LinkTo/env-mc-chem(98)17.
2. Organisation for Economic Co-operation and Development. *Advisory document of the working group on GLP: the application of the principles of GLP to* in vitro *studies.* Paris: OECD; 2004. (OECD Series on principles of GLP and Compliance Monitoring, No. 14, ENV/JM/MONO(2004)26).
3. Cooper-Hannan R, Harbell JW, Coecke S, Balls M, Bowe G, Cervinka M, Clothier R, Hermann F, Klahm LK, de Lange J, Liesbsch M, Vanparijs P. The principles of good laboratory practice: application to *in vitro* toxicology studies. *ATLA* 1999;27:539-77.
4. Organisation for Economic Co-operation and Development. *Bacterial reverse mutation test*. Paris: OECD; 1971. (OECD Guideline for Testing of Chemicals, No. 471).
5. Organisation for Economic Co-operation and Development. In vitro *mammalian cell gene mutation test.* Paris: OECD; 1971. (OECD Guideline for Testing of Chemicals, No. 476).
6. Organisation for Economic Co-operation and Development. *In vitro mammalian chromosome aberration test*. Paris: OECD; 1971. (OECD Guideline for Testing of Chemicals, No. 473).
7. Balls M, Blauboer BJ, Fentem JH, Bruner L, Combes RD, Ekwall B, Fielder RJ, Guillouzo A, Lewis RW, Lovell DP, Reinhardt CA, Repetto G, Sladowski D, Spielmann H, Zucco F. Practical aspects of the validation of toxicity test procedures. *ATLA* 1995;23:129-47.
8. Harbell JW, Southee JA, Curren RD. The path to regulatory acceptance of *in vitro* methods is paved with the strictest scientific standards. In: *Animal alternatives, welfare and ethics.* van Zutphen LFM, Balls M (Ed.). Amsterdam, The Netherlands: Elsevier; 1997. p. 1177-81.
9. Organisation for Economic Co-operation and Development. *Consensus document of the working group on GLP: the application of the OECD principles of GLP to the organisation and management of multi-site studies.* Paris: OECD; 2002. (OECD Series on principles of GLP and Compliance Monitoring, No. 13, ENV/JM/MONO(2002)9).
10. Wagner W, Horn P, Castoldi M. Diehlmann A, Bork S, Saffrich R, Benes V, Blak J, Pfister S, Eckstein V, Ho A D. Replicative senescence of mesenchymal stem cells: a continuous and organized process. *PloS One* 2008;3:1-12.
11. Kumar A, Ali A, Yerneni LK. Tandem use of immunofluorescent and DNA staining assays to validate nested PCR detection of mycoplasma. *In Vitro Cell Dev Biol Anim* 2008;44:189-92.
12. Organisation for Economic Co-operation and Development. *Consensus document: quality assurance and GLP.* Paris: OECD; 1999. (OECD Series on principles of GLP and Compliance Monitoring, No. 4 (revised), ENV/JM/MONO(99)20).
13. Organisation for Economic Co-operation and Development. *Consensus document: the application of the GLP principles to short term studies.* Paris: OECD; 1999. (OECD Series on principles of GLP and Compliance Monitoring, No. 7 (revised), ENV/JM/MONO(99)23).