

Chapter 4. Apparatus, material and reagents

Key message: *Apparatus, including validated computerised systems, should be regularly maintained, calibrated and validated (if required). Material and reagents should be purchased from well-established sources to ensure the integrity and reliability of the in vitro method results.*

Key content: *Quality requirements for equipment, material and reagents (e.g., use of serum, alternatives to the use of animal sourced serum, antibiotics, special media, certificate of analysis, stability and traceability) are detailed.*

Guidance for improved practice: *By detailing the diversity in availability of in vitro related materials and reagents the reader can identify for his/her work their advantages and limitations.*

Recommendations *are given to reduce experimental variability and increase within- and between-laboratory reproducibility by understanding the material and reagents you are working with and to take care that calibrated apparatus performance checks are carried out and operation limits are set adequately.*

4.1. Apparatus

Apparatus, including computerised systems, used for the generation, storage and retrieval of data, and for controlling environmental factors relevant to a study should be suitably located and of appropriate design and adequate capacity. Apparatus should be periodically inspected, cleaned, maintained, and calibrated according to Standard Operating Procedures (SOPs) and records of these activities should be maintained (OECD, 1998^[1]). In general, all apparatus used should be operated by trained staff.

The routine requirements for apparatus used in a Good Laboratory Practice (GLP) environment apply equally to apparatus used for *in vitro* studies (OECD, 2004^[2]). However to ensure the integrity and reliability of some results, certain equipment such as microbalances, plate readers, centrifuges, micropipettes, laminar air flow biological safety cabinets, fridges and freezers, water baths, and incubators should be regularly maintained, monitored and calibrated (if applicable). Calibration standards should be traceable to international standards if possible. For each type of equipment, critical parameters (e.g., supply of gases for mass spectrometry, liquid nitrogen levels in storage containers, low temperature storage (fridge/freezers), temperature and CO₂ levels in incubators) should be identified as requiring continuous monitoring or the setting of limit values together with installation of alarms.

Centrifuges which are routinely used in cell and tissue culture work (subculturing, cryopreservation, etc.) may produce aerosols and therefore it is important to consider models that have sealed buckets. Ideally, one should consider working with models where the condition of the load can be observed without opening the lid. Besides the containment issues for centrifuges it is necessary to specify centrifugation speeds as G-force (g) rather than Revolutions Per Minute (RPM) (unless the rotor radius is stated), incubation conditions, time and volumes of centrifugation with tolerances when relevant, and any other information that enables the accurate reproducibility of procedures. In addition, procedures should be established on cleaning, including cleaning frequency of buckets, caps, adapters, rotor, and bulkhead so as to reduce the possibility of contamination of cultures. Procedures should also be established regarding potential exposure and how to respond in case of an emergency (e.g., broken tubes).

Working with cell and tissue culture requires a strictly controlled environment for cell growth. This is achieved using specialised incubators which provide the correct growth conditions (temperature, humidity, CO₂ levels), which should be controlled (and logged) on a regular basis. Incubators that use a nebuliser to deliver humidity are preferred to older models which use a water pan/basin for the same purpose. This combination of high humidity and temperature increases the risk of bacterial or fungal contaminations and therefore care should be taken when using a water pan/basin equipped incubator. If using an incubator with a water pan/basin, sterile distilled (or equivalent) water should be used, and antifungal or bactericidal agents can be added to the water pan/basins to reduce the risk of bacterial and fungal growth. However, any possible impact due to the use of these agents on the *in vitro* method to be carried out should be checked and documented. Good practice is to avoid contact of any bactericidal/fungicidal agent with the cells and/or reagents used in tissue or cell culture. Another option to reduce the risk of microbial contamination is to use copper-coated incubators which are now available. Incubators with self-sterilising cycles may also be used, although this does not replace regular cleaning and maintenance.

Similarly, water baths used to thaw and/or to warm up stored solutions like medium and frozen stocks, or to defrost vials of cryopreserved cells and tissues, carry a high risk of introducing contamination. Sterile or deionised water should be used and the water should be regularly changed. It is good practice to carefully wipe down media bottles and/or cryopreserved vials with paper towels wetted with 70% (isopropyl) ethanol or other sterilising solutions before their transfer to a Biological Safety Cabinet (BSC). The use of bactericidal and fungicidal agents in water baths can aid in the control of contamination, but their impact on the test system should be checked and documented, and avoided where possible. Bead baths may also be used so as to reduce cross-contamination that may be more likely in water baths, especially when using tubes that may not be water-tight. Bead baths sometimes take longer to get up to the set temperature and accidental spills or contamination requires thorough washing and decontamination of the beads.

A BSC (Section 3.2.3) should be considered as a critical piece of equipment for cell and tissue culture work, since, when it is used correctly (Section 3.2.3), it ensures a clean working environment providing protection for both the operator and for cells/tissues and other materials and reagents. BSCs require regular service and maintenance such as integrity testing of High-Efficiency Particulate Air (HEPA) filters, testing of airflow velocity profile and testing of non-viable particle counting to make sure the cabinet is fully functional. Laboratory personnel must be fully trained in how to work within the BSC so to maintain aseptic culture technique.

For equipment such as refrigerators and freezers, temperatures should be checked regularly and preferably logged, e.g., using data loggers to record the temperature at set intervals. In addition to the regular recording of temperatures, an alarm system to alert staff when acceptable operating limits are exceeded is desirable, and a backup system should be in place, such that materials may be transferred from one fridge/freezer to another, in case of malfunction or for cleaning.

Acceptable operating limits should be set, monitored and recorded for all measuring equipment. Equipment should be fit for purpose with respect to sensitivity and selectivity. Equipment used to perform measurements should be calibrated¹ or verified², usually described in the facility SOP(s), at specified intervals or prior to use. As an example, pipettes or micropipettors may need to be checked more frequently than centrifuges. If during the checking errors are encountered, the pipette may need to be adjusted and recalibrated to ensure it meets the stated acceptable operating limits. A maintenance schedule should be implemented detailing the frequency of maintenance (e.g., yearly) of all equipment.

When pipetting volatile/viscous liquids or suspensions, it is strongly recommended to use positive displacement pipettes. Certain chemicals may exhibit non-specific adsorption to the plastic tips of pipettes and the use of low-binding materials (including glass) or acoustic droplet ejection (Ekins, Olechno and Williams, 2013^[3]; Grant et al., 2009^[4]) and can be utilised to alleviate these issues.

It may be necessary to have separate procedures for regular checks and complete calibration depending on the frequency of use and the criticality of the instrument. The frequency of checking may be extended if historical data shows low failure rates. When equipment such as a pipette is out of specifications during a calibration, it is important to determine how to interpret data that have been generated since the most recent successful calibration and determine the impact of the potential deviation on the outcome of the study. Therefore, it is crucial to record every piece of equipment, uniquely identified, that has been used during the performance evaluation of an *in vitro* method. In general,

facility practices should ensure that equipment is within specifications before the start of a study and throughout the experimental phase to avoid rejection of the *in vitro* study data.

Complex instrumentation, i.e. computerised systems including robotic systems, should be formally validated prior to use in a GLP study and procedures should be established to ensure that these systems are suitable for their intended purpose and are operated and maintained in accordance with the Principles of Good Laboratory Practice (OECD, 2016_[5]; OECD, 1998_[11]). The level of validation will depend on the systems complexity and its intended use and usually includes documentation of User Requirement Specifications (URS), a validation plan and report, user acceptance testing and reporting. More complex systems require in addition formally documented qualification of the system via Design Qualification (DQ), Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ), where the IQ and OQ may be performed by the supplier/manufacturer. Whatever approach is taken, it should be justified by a documented risk assessment (FDA, 2001_[6]; OECD, 2016_[5]). The Official Medicines Control Laboratories (OMCLs) guidelines for qualification of equipment may be helpful in designing the validation for a given computerised system (OMCL, 2011_[7]).

To enable broader use of a new method, successful transfer to a range of equipment (if applicable) and different laboratories should be demonstrated. This increases the robustness of the method. To increase transferability, preference should be given to the use of generally widely available equipment. In addition, the impact of the use of a certain type or brand of equipment on the outcome of the individual assays or the overall *in vitro* method needs to be determined. An *in vitro* method should specify the requirements the equipment should meet to be used for the specific method.

4.2. Materials and reagents

Reagents are often selected on the basis of historical use or from references in relevant documents associated with regulatory accepted *in vitro* methods (e.g., validation reports, *in vitro* method SOPs). It is good practice to have procedures for maintaining and controlling laboratory stocks of reagents such as maintaining a minimum stock level for critical reagents.

The *in vitro* method should use reagents from well-established sources (to avoid as much as possible labour intensive control checks), preferably certified suppliers. OECD recommends that suppliers implement the International Standard ISO 9001, and particularly Part 1 - Specification for Design/Development, Production, Installation and Servicing (OECD, 2000_[8]). Nowadays most suppliers have adopted manufacturing practices which comply with formal national or international standards, such as ISO 9001. Identification and qualification of alternate suppliers for critical reagents and materials is also recommended.

Preparation of reagents should be tracked (e.g., by use of logbook or electronic record(s)) to retain information such as the supplier, catalogue number, batch/lot numbers (if appropriate), dates of preparation and expiry, and the names of the operator involved in the preparation. For both reagents and reagents mixtures, the container should be inert and not affect the stability of the substance or mixture. Attention will also need to be given to the suitability of reagents and to the safety and ethical provenance of cells (Coecke et al., 2005_[9]; Pamies et al., 2016_[10]).

Labelling of reagents should be defined in a procedure (SOP) and should include identity, concentration (if appropriate), expiry date and specific storage instructions. The expiry date may be extended on the basis of documented evaluation or analytical analysis (OECD, 1998_[1]).

Storage should be done according to the manufacturer's specifications as detailed in the Certificate of Analysis (CoA) or product information sheet. Some solutions, e.g., solutions which require storage below 0°C, may be aliquoted in order to minimise the number of times a bottle is opened and thus minimise the risk and spread of contamination and avoid repeated freeze/thaw cycles. When reagents need to be thawed and possibly frozen again, it is recommended to determine the number of freeze/thaw cycles that the reagents can withstand (EMA, 2011_[11]; FDA, 2001_[6]; Viswanathan et al., 2007_[12]); (FDA, 2001_[6]). Stability of aliquots should be verified in the laboratory performing the *in vitro* method and should not be based solely on literature data.

Even when reagents are sourced from a reputable supplier, it remains important to assure the stability of the reagents during shipment conditions, in addition to the storage. For example, reagents shipped frozen should arrive frozen and this should be documented on the receiving document. The presence of a data logger is the best practice in these cases.

Quality checks, if required, should be performed according to pre-defined procedures described in SOPs. Normally, stability of the analyte in the studied matrix is evaluated using at least triplicate samples of the low and high concentrations, which are analysed immediately after preparation and after the applied storage conditions that are to be evaluated. The thawed samples are analysed against a calibration curve, obtained from freshly prepared calibration standards, and the obtained concentrations are compared to the nominal concentrations. The deviation should be within previously established acceptance criteria (usually $\pm 20\%$) (EMA, 2011_[11]). It is absolutely necessary that the number of cycles in the freeze/thaw stability evaluation should equal or exceed that of the freeze/thaw cycles of study samples.

4.3. Use of media in cell culture

Depending on the circumstances, the basal culture medium can be animal serum-supplemented (as in traditional cell culture methods) or serum-free, but supplemented with additives necessary for obtaining satisfactory cell proliferation and production, or for maintaining a desired differentiation status. Many slightly different formulations exist under the same general medium names, such as Minimum Essential Medium (MEM), and even subtle changes in the medium formulation can substantially alter the characteristics of certain cells and tissues. In many cases, these variations are deliberate for specific applications. Therefore, the medium to be used should be precisely specified, and it is essential to check that new supplies of medium meet the required specifications (Coecke et al., 2005_[9]). If a medium other than that recommended/indicated by the cell provider is used then the justification should be documented and the effect on baseline cell properties should be determined and provided with the final data.

4.3.1. The use of animal sourced serum in cell culture

Serum is a complex mixture, introducing undefined components into the medium. Many of these substances have not yet been identified, and in many cases the effects on cultured cells are as yet unclear. Animal serum can be derived from adult, new born or foetal sources, but typically less than 24 months old animal sources should be used³ (Festen,

2007_[13]). Bovine sera are most commonly used and Foetal Calf Serum (FCS)⁴ has become the standard supplement for cell culture media in the last few decades.

In vitro method developers must determine serum specifications that meet their particular needs and match the natural behaviour of the cells as much as possible, including defining the maximum acceptable levels of serum components, such as immunoglobulins (which may have inhibitory effects), endotoxins (indicative of bacterial contamination, but are also powerful cell mitogens), and haemoglobin (indicative of haemolysis during clotting). Furthermore, if the quality of the serum is deemed critical to the performance of the method, more rigid testing requirements will apply and should be specified in the respective Test Guideline (TG).

Ideally, sera should be obtained from vendors that can provide traceability certification from industry bodies such as the International Serum Industry Association (ISIA). Vendor's documentation, usually in the form of a CoA, generally include country of origin and traceability information, filtration steps used in serum processing, sterility testing, screening for mycoplasma and virus, endotoxin, lot number, storage conditions etc. (Sadeghi et al., 2017_[14]). Test facilities rely on the documentation the supplier provides, including the compatibility of different lots/batches of serum.

Batches of serum can differ dramatically in their ability to support the growth of cell lines due to variation in the concentration of growth factors and hormones, therefore, new batches should be tested on the appropriate cell line(s) for cell attachment, spreading, cloning efficiency, growth rates and activity in functional assays (Geraghty et al., 2014_[15]). Testing of serum batches will ensure in-house reproducibility. Some facilities, based on experience with specific test systems, do not always perform full additional batch testing however this should be judged on a case by case basis.

Serum can interfere with phenotypic cell stability, and may influence experimental outcomes. Serum can suppress for instance embryonic stem cell differentiation and tissue formation. The use of FCS can possibly lead to unexpected or undesired outcomes, e.g., FCS can inhibit transforming growth factor (TGF)- β 1-induced chondrogenesis in fibroblast-like type-B synoviocytes (Bilgen et al., 2007_[16]). FCS compared to autologous (human) serum has been found to induce a more differentiated and less stable transcriptional profile in human bone marrow mesenchymal stem cells, particularly at late passages, as shown by analysis of genome-wide microarray analysis (Shahdadfar et al., 2005_[17]).

Cell lines which have been derived or cultured long-term in serum-containing media may become dependent on the multitude of growth factors present and may experience a phenotypic drift upon abrupt serum withdrawal. This may manifest as growth arrest or activation/inactivation of various signalling pathways. These effects can be overcome by adaptation to serum-free culture conditions (Section 4.3.3) using specific protocols (Beltran et al., 2014_[18]; Leong et al., 2017_[19]; Sinacore, Drapeau and Adamson, 2000_[20]) for a gradual weaning of cells (van der Valk et al., 2010_[21]).

4.3.2. The use of animal sourced serum in cell culture for endocrine activity

To study the effects of chemical substances that may have endocrine activity (e.g., steroid hormones), endogenous hormones, growth factors and cytokines are removed by charcoal stripping of serum. If FCS is required in Endocrine Active Substances (EAS) *in vitro* methods, it is necessary to use Dextran-Coated-Charcoal-treated Foetal Calf Serum⁵ (DCC-FCS) when performing these tests. DCC-FCS as a basic component of cell culture

medium has become the standard supplement and has been listed in several OECD TGs, e.g., TG 455 and TG 458.

A 2005 study found that DCC-FCS affected the commitment of osteoprogenitor KS483 cells, strongly promoting adipogenesis compared to normal FCS containing medium, which drives KS483 cells to differentiate into only osteoblasts (Dang and Lowik, 2005_[22]). This suggests possible unpredictable effects of DCC- FCS on progenitor cell differentiation.

4.3.3. Alternatives to the use of animal sourced serum

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. In 2008 the ECVAM Scientific Advisory Committee (ESAC) stated that "*for methods forwarded to ECVAM for validation/prevalidation where [the use of non-animal alternatives to serum] is not fulfilled a justification for future use must be provided, including measures taken to seek non-animal alternatives to [FCS]*"⁶. The drawbacks of using FCS and the recommendation to replace it with available chemically defined serum free media is already discussed in the GCCP guidance document issued by EURL ECVAM (Coecke et al., 2005_[9]). Furthermore, it is recommended to develop new *in vitro* methods with a serum-free, chemically-defined medium, to avoid potential sources of uncertainty that may be introduced by using animal serum (Jochems et al., 2002_[23]; Pamies et al., 2016_[10]).

Serum-free media (Table 4.1) are thought to circumvent many of the drawbacks of using FCS including the batch to batch variability issues associated with serum and offer better reproducibility and the potential for selective culture and differentiation of specific cell types (Geraghty et al., 2014_[15]). Nevertheless, serum-free compositions may still need to be validated and monitored similarly to serum containing media as they are often not completely chemically defined. A source of a range of commercially available serum-free media for cell-culture, as well as medium compositions obtained from scientific literature, is provided by the 3Rs-Centre ULS in collaboration with Animal Free Research UK (FCS-free database⁷).

Table 4.1. Serum-free media

Media	Description
Serum-free	Does not require supplementation with serum, but may contain discrete proteins or bulk protein fractions (e.g., animal tissue or plant extracts) and are thus regarded as chemically undefined (see: chemically defined media).
Protein-free	Does not contain high molecular weight proteins or protein fractions, but may contain peptide fractions (protein hydrolysates), and are thus not chemically defined. Protein-free media facilitate the down-stream processing of recombinant proteins and the isolation of cellular products (e.g., monoclonal antibodies), respectively.
Animal-product-free	Does not contain components of animal or human origin. These media are not necessarily chemically defined (e.g., when they contain bacterial or yeast hydrolysates, or plant extracts).
Chemically defined	Does not contain proteins, hydrolysates or any other components of unknown composition. Highly purified hormones or growth factors added can be of either animal or plant origin, or are supplemented as recombinant products (see: animal-product-free media).

Source: (van der Valk et al., 2010_[21])

The use of human serum was originally restricted to specialised applications (Coecke et al., 2005^[9]). However due to better quality controls, including documentation to demonstrate origin and viral safety, human serum has become more widely used (Blázquez-Prunera et al., 2017^[24]; Dessels, Potgieter and Pepper, 2016^[25]; Even, Sandusky and Barnard, 2006^[26]; Gstraunthaler, Lindl and van der Valk, 2013^[27]); (Dessels, Potgieter and Pepper, 2016^[25]; Even, Sandusky and Barnard, 2006^[26]; Gstraunthaler, Lindl and van der Valk, 2013^[27]; Jochems et al., 2002^[23]; Kanafi, Pal and Gupta, 2013^[28]); and has been shown feasible by adapting the KeratinoSensTM skin sensitisation test to xeno-free cell culture (Belot et al., 2017^[29]). The same critical points, e.g. batch-to-batch variability, as for any serum-derived products hold true.

Human platelet lysates (hPLs) have been proposed as an alternative growth supplement to FBS. hPLs are the result of freeze-thawing platelet concentrates and contain several growth factors (Bieback et al., 2009^[30]). Platelet concentrates, typically products manufactured for transfusion purposes, can be used as a cell culture supplement after the shelf life of the donation program has expired. As these programs are managed by certified blood donation centres, hPLs are therefore obtained from safe and clinically tested sources. hPLs have now been successfully used in several applications such as growth and maintenance of renal epithelial cell lines and human mesenchymal stromal cells, and storage of human tissues for patient related treatment (van der Valk, 2018^[31]). hPLs cannot be considered a defined supplement, though.

Other serum free media can include poorly defined supplements such as pituitary extracts, chick embryo extracts, bovine milk fractions or bovine colostrum. Furthermore, some so-called ‘defined’ media contain complex serum replacement mixtures including chemically undefined agents. Notably B27 and its alternative NS21 used in the culture of neural cells contain bovine serum albumin and transferrin which can exhibit batch to batch variation in biological activity (Chen et al., 2008^[32]). Therefore, it may be useful to carry out pre-use testing on new batches of reagents which could demonstrate variability that cannot be foreseen from the manufacturers’ information. Another example of an essential component prone to batch to batch variability is the so-called ‘basement membrane extract’, purified from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells and marketed under various trade names.

Chemically-defined media are cell-type specific, in contrast to FCS (van der Valk, 2018^[31]), and have to be selected and optimised for the selected cell type (Price, 2017^[33]) These media are commercially available for many cell types⁸, but the formulations of these are generally not released because of proprietary reasons. Therefore, it is important to check before use what cell lines a chemically-defined medium is available or optimised for. It is also possible to develop a chemically-defined medium and adapt cells as defined by van der Valk (van der Valk et al., 2010^[21]). Applying factorial design approaches have been shown to minimise the screening time, allow prediction for best medium formulation and can be used as a high-throughput medium optimisation platform (Zhao et al., 2017^[34]).

Serum-free medium formulations for culturing of stem cells, such as human Embryonic Stem Cells (hESCs) and human induced Pluripotent Stem Cells (hiPSCs), show promise for applications in toxicology, regulatory testing and biomedical research (Colatsky et al., 2016^[35]). A recent study (van Velthoven et al., 2017^[36]) indicates that stem cells *in vivo* may have a very different gene expression profile *in vitro* which should be taken into consideration when conducting *in vitro* studies examining stem cell function. Both hESCs and hiPSCs are often maintained on inactivated mouse embryonic fibroblasts or under

feeder-free conditions (using extracellular matrices) in chemically defined, serum-free media, in order to avoid the presence of undefined or unknown serum components (which may compromise the differentiation towards desired cell lineages) and the risk of contaminations from pathogens (e.g., mycoplasma, viruses, and prions) (Pistollato et al., 2012_[37]; Yamasaki et al., 2014_[38]).

4.3.4. The use of phenol red in cell culture

Phenol red is used in the cell culture as a convenient way to rapidly check on the health of cell or tissue cultures. In the initial cell culturing stage, a small amount of phenol red is often added to the cell culture medium. Under normal conditions most living cells or tissues prosper at a near-neutral pH, i.e. a pH close to 7, and the culture medium has a pink-red colour as an indicator colour. Under abnormal conditions, cellular waste products or contaminants will cause a change in pH, leading to a change in indicator colour.

Phenol red can interfere with some spectrophotometric and fluorescent assays, and it is also weakly estrogenic. To avoid the possibility of interference with specific assays, it is therefore recommended that phenol red-free medium be used.

4.4. The use of antibiotics in cell culture

Routine cell and tissue culture according to GCCP (Coecke et al., 2005_[9]; Geraghty et al., 2014_[15]; Stacey and Davis, 2007_[39]) 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 (OECD, 2004_[2]) 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. Not surprisingly, antifungal agents, being directed at higher order, eukaryotic microorganisms, are likely to be more toxic to animal cell cultures. In addition, antibiotics often make it more difficult to detect microbial contamination. Given these obvious contra-indications, the use of antibiotics in cell and tissue culture should be focused in two areas: a) protection of materials at high risk of contamination such as tissues, organs and primary cultures in cases where sterility cannot be guaranteed; and b) the positive selection of recombinant cell clones based on the expression of antibiotic resistance genes (Coecke et al., 2005_[9]). If antibiotics are needed, a justification for the use of antibiotics in the procedure is recommended.

4.5. Additional media components

Some media components are heat labile (e.g., L-glutamine), sensitive to light (e.g., retinoic acid) or have a limited half-life in diluted state or at high ionic strength, such as in prepared media (e.g., epidermal and fibroblast growth factors). These issues are best addressed by preparing a small volume of media necessary to cover the period of stability of the most sensitive component and discarding bottles after a set time period. Appropriate size aliquots of those labile components may be frozen by an appropriate method for long-term storage. In this respect, stock solutions with low concentrations of protein aqueous growth factors may require the addition of albumin or other excipients to prevent adsorption to plastic and to increase stability in the frozen state. Stabilised forms of glutamine and retinoic acid are also available to avoid these issues.

In case culture media or other reagents have to be sterilised via heat or filtration, the impact of the procedure (e.g., comparison of doubling time to historical data) should be assessed and recorded. For example, heat sterilisation may result in degradation (or denaturation) of one or more of the components and filtration can remove individual and/or essential components (e.g., Fe^{2+} or Fe^{3+} iron products that enhance growth of mammalian eukaryotic cells in serum-free cultures).

4.6. Dedicated media for particular cell lines

Different cell types or tissues need to be cultured in media containing various components at different concentrations to allow optimal growth. Although certain cell lines may be grown in media with the same composition, sharing media between cell lines increases the risk of cross-contamination. Therefore, each cell line should be cultured with separate dedicated media, which must not be shared with other cell lines. It is important to note that different media types are not only used for different cell cultures, but also for the same culture (e.g., when differentiating HepaRGs to hepatocyte-like cultures or primary lung epithelium cells in air-liquid interface culture different media are used in the differentiation procedures). Sharing media between laboratory personnel also increases the risk of contamination and cross-contamination and should be avoided.

Notes

1. Calibration, as used here, is a measurement against a known standard and may involve adjustment of the apparatus, which may or may not be described in the equipment manual.
2. Verified, as used here, is a confirmation that the device fulfils specified requirements where no adjustment is possible.
3. <http://www.thermofisher.com/it/en/home/life-science/cell-culture/mammalian-cell-culture/fbs/other-sera/bovine-serum.html>
4. Also known and available as Foetal Bovine Serum (FBS)
5. Also known as Dextran-Coated-Charcoal-treated Foetal Bovine Serum (DCC-FBS)
6. https://eurl-ecvam.jrc.ec.europa.eu/about-ecvam/archive-publications/publication/ESAC28_statement_FCS_20080508.pdf
7. <https://fcs-free.org/>
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