Chapter 3. Facilities

Key message: *In vitro cell and tissue culture facilities should be fit for purpose and a detailed understanding of the work flow for the in vitro method related processes is essential. The separation of specific laboratory functions and elements that can adversely impact in vitro method work need to be understood.*

Key content: *Elaborates safety, safety risk assessment and management including descriptions of Risk Groups and Biosafety Level requirements, proper facility design to ensure integrity of the cell and tissue cultures, the in vitro method itself and the resulting data.*

Guidance for improved practice: *This chapter describes guidance on level of separation to avoid cross-contamination and quarantine measures for new test systems. A flow diagram indicating movement of staff, materials and reagents, test systems and test and reference items, and waste collections shows what processes need to be separated.*

Recommendations *for classification of infective microorganisms, laminar flow biological safety cabinets and biosafety levels, are given.*

Facilities must be fit and suitable for the purpose of the work; that is, size, construction, and location should be appropriate, and the building should allow for the separation of activities.

Higher containment levels may be required depending on the biosafety risk level (Section 3.2.2) of the biological agents handled. If *in vitro* work is to be performed with test systems belonging to Risk Group III or IV (Section 3.2.1), which can cause severe human disease and may be a serious hazard to employees or spread to the community, then separated facilities, appropriate Biosafety Levels (BSLs) such as air filtration and negative pressure differences, will need to be maintained (Section 3.2.2). Risk Groups III and IV are more complex in complying with specific facility requirements and personnel skills. Therefore if possible, *in vitro* methods for regulatory use in human safety assessment should be developed to require mainly BSL 2 or less.

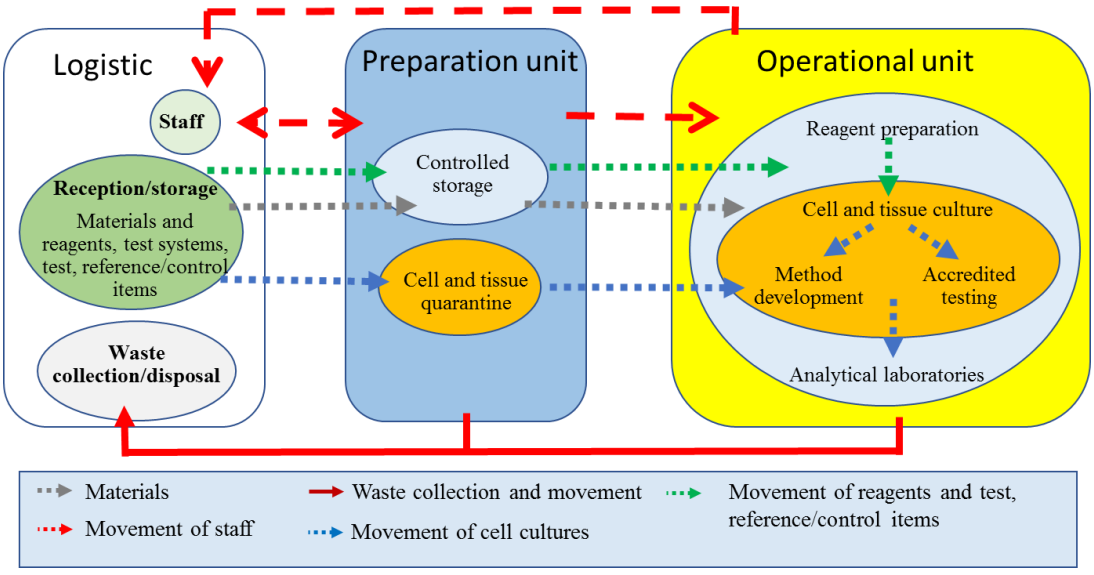
3.1. Facility design

When designing a new facility or modifying an existing facility, a safety risk assessment should be performed (Section 3.2), as safety should be included in the design phase. This is more critical for BSL 3 or 4 facilities (Section 3.2.2). It is important to understand the workflow for the intended processes and those aspects which could impact adversely on others, so that the facility design will facilitate smooth and safe laboratory procedures, storage and waste disposal.

Facilities should be designed or adapted to minimise the risk of errors (e.g. mix-ups) and to avoid (cross-)contamination which may adversely affect the quality of the work performed. Services (e.g., gas, electricity, liquid nitrogen) should ideally be accessible for routine maintenance to minimise interference in laboratory work. All the necessary permits should be in place before any activities are initiated. Finally, there should be dedicated areas for data storage and archiving. An Uninterruptible Power Supply (UPS) should also be available for all critical equipment, including reagent/sample storage[1] (especially critical for low-boiling point reagents) to ensure preservation in case of loss of power.

The types of laboratory functions, along with the flow of work and materials, outlined in Figure 3.1 are among those to be taken into consideration for separation (physical or process/training), that someone establishing or running a facility should be aware of. It may not be possible or acceptable to separate all functions. Other functions, specific to the type of work performed, may also need to be taken into consideration. It is wise to avoid physical contact between materials transfers and waste removal so that there is very low risk of contamination from waste affecting reagents, cultures and test materials.

Figure 3.1. Flow of staff and materials to show separation of processes



Note: It is recommended that each area have their own dedicated storage facilities so as to avoid mixing up test items and/or reagents.

As contaminated working surfaces can lead to microbial contamination or cross-contamination between test systems and pose a risk to the *in vitro* method quality, working surfaces should be easy to clean, resistant to acids, alkalis, solvents and disinfectants. There should be appropriate documented procedures for disinfection of work surfaces, safety cabinets and equipment.

Physical separation of pre and post Polymerase Chain Reaction (PCR) assay stages should be maintained to minimise contamination and cross-contamination (Section 3.3). Between these two areas the work flow should be unidirectional. Equipment, consumables and laboratory coats should each have a dedicated area. It is recommended that facilities performing PCR methods should be organised in four discrete areas[2]. Requirements may vary with assay format e.g., real time PCR does not require post-PCR analysis[3].

1. **Reagent preparation clean area** - air pressure should be positive.
2. **Nucleic acid extraction area** - air pressure should be positive. If chemicals are stored in this area appropriate facilities and storage requirements should be in place.
3. **Amplification area** - PCR machines are housed in the Amplification room. It may contain an area/cabinet with air pressure slightly positive for the nested PCR.
4. **Product analysis area** - air pressure should be negative.

Good Laboratory Practice (GLP) test facilities require archive(s) that should provide for the secure storage and retrieval of study plans, raw data, final reports, samples of test items and specimens (OECD, 2004[1]). Archive design and archive conditions should protect contents from untimely deterioration.

3.2. Safety, risk assessment and management

Countries or regions may have specific classification of microorganisms and/or other hazards, which should be consulted when performing the safety risk assessment.

A risk management approach should be used when introducing new processes or when modifying the design of the facility, so as to eliminate potential hazards prior to their introduction. Risk management is a process or method used to identify, evaluate and determine the appropriate way to deal with exposure to hazards and risk factors that have the potential to cause harm, and is an ongoing process that requires continuous review to ensure that the implemented control measures work as planned.

When planning a safety risk assessment all hazards should be considered, including physical, chemical, photic and biological hazards and should comply with all national and/or international legislation. The risk assessment should not be limited to just the laboratory, but should also consider the entire site and any possible risks to the environment, including waste disposal for any hazardous materials and again should comply with national laws.

Safety risk assessments should be performed by the individual(s) most familiar with the specific characteristics of the test systems being considered for use, the equipment, materials and reagents, the procedures to be employed, and the containment equipment and facilities available. Exposure to these hazards might be complex and may require specialist knowledge both in identifying and evaluating their associated risks and designing appropriate actions to avoid or minimise them.

The transport of dangerous items should also be addressed in the risk assessment, specifically what precautions to take in case of spillage. International transport, either by rail, air or road should comply with international norms, e.g., International Air Transport Association (IATA) and/or the Dangerous Goods Regulations (DGR).

Training of staff (Section 2.6) in preventative procedures such as the correct use of Biological Safety Cabinets (BSCs), aseptic techniques, use of personal protection equipment (PPE), waste disposal, etc., will not only ensure a safer working environment but will also benefit the quality of the work performed.

3.2.1. Risk Groups

In many countries biological agents are categorized in Risk Groups Figure 3.1 based on their relative risk. Most countries have national or local laws and regulations governing safety in the workplace. Many of these national regulations classify microorganisms based on the biological risks they present to human health and/or to the environment. While no agreed international classification scheme exists, the WHO formulated a set of minimum standards for laboratory safety detailing four risk groups, the last version having been published in 2003. Variation of these four risk groups have been implemented into national laws worldwide.

Table 3.1. Classification of infective microorganisms by risk group

Risk Group I	low individual and community risk	A microorganism that is unlikely to cause human disease or animal disease of veterinary importance.
Risk Group II	moderate individual risk, limited community risk	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread is limited.
Risk Group III	high individual risk, low community risk	A pathogen that usually produces serious human disease but does not ordinarily spread from one infected individual to another.
Risk Group IV	high individual and community risk	A pathogen that usually produces serious human or animal disease and may be readily transmitted from one individual to another, directly or indirectly.

Source: (WHO, 2004[2])

3.2.2. Biosafety Levels

The assignment of a biosafety level should take into consideration a multitude of factors, such as the microorganism or pathogenic agent used, the facilities available, the equipment practices and procedures and should not be just the automatic assignment according to the particular risk group (WHO, 2004[2]).

BSL prescribes procedures and levels of containment for the test systems and materials. Test facilities may be assigned to one of four BSL based on a safety risk assessment (Table 3.2).

Table 3.2. Summary of Biosafety Level requirements

	1	2	3	4
Isolation[a] of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation				
• inward airflow	No	Desirable	Yes	Yes
• controlled ventilating system	No	Desirable	Yes	Yes
• HEPA-filtered air exhaust	No	No	Yes/No[b]	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	Yes	-
Anteroom with shower	No	No	Yes/No[c]	Yes
Effluent treatment	No	No	Yes/No[c]	Yes
Autoclave				
• on site	No	Desirable	Yes	Yes
• in laboratory room	No	No	Desirable	Yes
• double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability[d]	No	No	Desirable	Yes

Source: (WHO, 2004[2])

Cell lines and primary tissues may carry a variety of different microorganisms or pathogens, which can potentially cause human disease, pose hazard to employees and

distort the *in vitro* method results. These should be handled at biosafety (hazard) level 2, unless known to be pathogen free including any likely serious human pathogens based on the origin and species of the material. This level of containment is also appropriate for monoclonal antibody-containing supernatants and cell homogenates. Access to level 2 facilities should be restricted to authorised personnel only, and specific safety risk assessment and training activities should be followed according to the national legislation on level 2 containment (Coecke, 2005[3]; Geraghty et al., 2014[4]).

3.2.3. Biological safety cabinets

BSCs are designed to use HEPA filters to capture particles 0.3 micron or bigger. Many are also designed to recirculate a high percentage of the filtered air within the cabinet (e.g., Class II A1 and A2 recirculate about 70% of the filtered air in the BSC) and to exhaust the remaining filtered air into the room or, if fitted, to an exhaust system. When handling toxic chemicals in a BSC it is critical to know the percentage of air exhausted into the room, due to the possibility of personnel exposure, and also the percentage recirculated in the BSC as it could potentially affect the test system and therefore the results generated with the *in vitro* method.

Chemical fume hoods, on the other hand, are designed to capture, contain, and exhaust hazardous fumes generated inside the hood, and should be used to handle and prepare hazardous chemicals whenever possible. When handling highly toxic materials the use of a glove box may be preferred (consult the facility's chemical risk assessment and/or the suppliers safety data sheets for the correct handling requirements). If a hazardous chemical is to be used in a BSC, the BSC should be equipping with an active carbon filter on the hood exhaust. The quantity of hazardous chemical used must be limited and where possible the pure hazardous chemical should not be handled inside the BSC.

Most commercially available BSCs are certified (e.g., the public health and safety organization NSF International/The American National Standards Institute ANSI 49 - 2012, Biosafety Cabinetry: Design, Construction, Performance, and Field Certification, BS/EN 5726 Microbiological safety cabinets) for the stated classification. This certification should also be confirmed once the cabinet has been installed in the facility so as to guarantee its proper functioning and regular testing performed to assure correct ongoing function.

A commonly accepted classification based on their containment capabilities and performance attributes has been adopted by most manufactures (Table 3.3). At a minimum, all cell and tissue work should be performed in a Class II biological safety cabinet as even screened tissues or cell cultures may carry infectious agents not covered by virological screening. Class I BSC, where the airflow is directed inward into the cabinet, provides protection for personnel and the environment but not for materials or work inside the cabinet, as it does not prevent contact with airborne contaminants that may be present in laboratory air. Class II BSC, often referred to as vertical laminar flow cabinets due to a unidirectional HEPA-filtered air stream that descends downward, provide protection for the personnel, the environmental and for the work performed inside the cabinet (Table 3.3). For guidance in use of Class II cabinets refer to the Good Cell Culture Practice (GCCP) principles (Coecke, 2005[3]).

Table 3.3. 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

If microscopes or other equipment are to be installed in a BSC, the cabinet should be checked for flow disruption so as to maintain the correct functionality of the BSC.

Splashes and aerosols carry contamination and infection risks, which not only endanger the operator but may also compromise the integrity of the *in vitro* method (i.e., cross contamination of cell lines or introduction of adventitious agents) results. Therefore, all procedures should aim at minimising aerosol production. Any procedures likely to produce aerosols should be contained (e.g., using a BSC) or the material should be rendered harmless.

3.2.4. Waste disposal

Prior to introducing new or modifying existing procedures it is necessary, and often required by law, to carry out a safety risk assessment which will include the assessment of any potential risks related to the waste generated. For most commercially acquired chemicals and reagents the suppliers' documentation will enable rapid assessment of potential associated risk. For test systems acquired from commercial cell providers the provided documentation may also be used to facilitate the risk assessment; however, for test systems obtained from another laboratory the documentation provided, if any, will rarely be sufficient, placing an extra burden on the facility. It is important that decontamination procedures are also put in place and are tested for their efficacy against those microorganisms likely to be present. Laboratory generated waste should be disposed of on a regular basic and not allowed to build up in the facility. The flow of waste removal within the facility should be such as to minimise potential secondary contamination.

3.3. Strategies to avoid cross-contamination

It is the responsibility of all laboratory staff to ensure that the correct workflow is followed and appropriate training should be given to the personnel regarding the necessary precautions to minimise contamination and cross-contamination, e.g., training on the use of aerosol-free/aerosol filter pipet tips when working with PCR assays.

Measures should be taken to ensure adequate separation of different biological agents and studies taking place in the same physical environment (OECD, 2004[1]). The integrity of each test system and study should be protected by spatial or temporal separation from other studies and test systems to avoid potential cross-contamination and mix-up. The flow of materials, staff and waste can be an important factor in controlling these issues and Figure 3.1 gives an illustration of how this may be applied.

Tissues and cells from different studies can be kept in the same incubator provided that they have the same incubation temperature requirements, are labelled appropriately, are spatially separated and none of the test items or solvents used are volatile enough to cause

contamination. Tissues and cells from different species or *in vitro* methods where yeast and bacteria are used require a higher level of separation. The most important issue here is to separate the areas used for cell culture/tissue and microbiological culture and that adequate care (e.g., use separate protective clothing) is taken not to carry over contamination from one area to the other, which would ideally be described in a SOP. Other degrees of separation may be achieved using the specific requirements described elsewhere for quarantine of untested material.

Temporal separation of test systems is possible in biological safety cabinets by handling only one test system at a time. Before introducing a new test system the cabinet working surfaces and related equipment should be cleaned and decontaminated, for example by cleaning with 0.5% solution of hypochlorite (approx. 5000 ppm free chlorine) followed by 70% isopropyl alcohol and then wiping with sterile wipes. The cabinet may then be exposed to UV light, if appropriate.

Rooms and areas used for preparation and mixing of test and reference items with vehicles should allow for aseptic working conditions in order to minimise the risk of microbial contamination of the test system.

When performing molecular biology techniques and especially PCR-based assays, which are high sensitivity methods, extreme care should be taken in facility design (Section 3.1) and operation. False-positive results can originate, for example, from sample-to-sample contamination from carry-over of nucleic acid from previous amplification of the same or similar target. Cloned DNA or virus-infected cell cultures may represent other source of contamination[4].

A major source of PCR contamination is aerosolised PCR products (Scherczinger et al., 1999[5]). Once these aerosols are created, being small, they travel and easily spread all over benches and equipment, where they can find their way into a PCR reaction and become amplified. Laboratories exclusively performing real-time PCR and properly discarding all amplified products without opening the reaction tubes or using sealed plates are less liable to contamination and could therefore be dispensed from the follow-up measures.

A no template control and a reverse transcription negative control should always be included in the PCR reaction test runs to exclude contaminations in reagents, in the work environment etc. When performing real time PCR, the use of dUTP in place of dTTP in the dNTP mix is recommended, in this way, all amplicons generated will have dUTP incorporated in them. In the future, if that amplicon becomes the source of contamination, using the enzyme Uracil-DNA-glycosylase prior to PCR specifically targets dUTP-containing DNA, resulting in excision of uracil, and prevents PCR contamination by a previous amplicon. The excision of uracil prevents the amplicon amplification by creating abasic sites in the amplicons. The abasic sites do not serve as good DNA templates for Taq polymerase. Therefore, the contaminated amplicons are prevented from being amplified further (Nolan et al., 2013[6]; Taylor et al., 2010[7]).

It is recommended to colour code racks, pipettes and laboratory coats in the different areas so to be able to easily monitor their movement between the different areas. Powder-free gloves should be used throughout the process in all the different areas as the powder on powdered gloves might affect the assay outcome/performance. It is particularly important to always use powder-free gloves in the pre-PCR area, as the pre-PCR area is prone to contamination by RNases.

The reagent preparation clean room should be free from any biological material such as DNA/RNA, cloned material, etc. Primers and reagents aliquoting is recommended to minimise contamination consequences. To ensure clean areas are kept free of amplicon at all times, there should be no movement from the dirty area to the clean area. If under extreme circumstances a consumable or reagent needs to be moved back it must be thoroughly decontaminated with bleach and ethanol. Returned racks should be soaked in a 0.05% solution of hypochlorite overnight before soaking in distilled water and placing in the clean area. To ensure minimal movement between areas during the running of molecular assays, it is optimal to have dedicated storage (freezer, fridge and room temperature) for each area. Room air pressure should be positive.

In the **nucleic acid extraction room/area** samples are processed, reverse transcriptase step of RT-PCR are performed and DNA or cDNA, and positive controls are added to the PCR reaction mix (prepared in the reagent preparation clean room).

Post-PCR manipulations such as agarose gel electrophoresis are performed in the P**roduct analysis room/area.** It is thus a contaminated area and therefore no reagents, equipment, pipettes, coats, etc. used in this room should be used in any other PCR areas. Bench areas should be wiped daily with hypochlorite solution following use and contaminated areas should be additionally decontaminated with ultra-violet radiation if available. Hypochlorite solutions containing more than 500 ppm available chlorine are corrosive to some metals and alloys and should not be applied to stainless steel (types 304/347, 316 and 400 series) as it may lead to corrosion with repeated use. It is recommended for personnel working with post-PCR assay stages not to work with pre-PCR parts later the same day. Monitoring of viable and non-viable particles of critical equipment surfaces and air flow within these areas/rooms may also be beneficial in controlling contamination.

3.4. Air handling, water supply, environmental control, heating and cooling

Air handling systems should be operated to ensure that the correct environment is maintained for the type of work conducted in the laboratory. These systems should be subject to regular maintenance and serviced by qualified personnel and records of maintenance, including modifications, should be retained to demonstrate appropriate upkeep and function. Where the *in vitro* work involves the use of human pathogens, the laboratory should operate with specific trained personnel, using biosafety level 3 or 4 and the room should be kept at negative pressure to guard against infection spread. When High-Efficiency Particulate Air (HEPA) filters are used in differential pressure isolation rooms, the filters and their fittings and seals need to be thoroughly examined and tested at regular intervals (e.g., annually). Decontamination should be carried out before servicing is carried out. Air handling systems should also be designed to account for exhaust air from the Class II biological safety cabinets to be vented outside.

Cell culture work requires cell/tissue culture grade water, which is usually deionised using reverse osmosis membrane separation, followed by passage through a series of carbon and micropore filters eliminating organic materials and pyrogens. Tissue culture grade water should be controlled for pH, conductance and total organic carbon. Note that pyrogens can be deleterious to cell cultures at concentrations below the level of detection for organic carbon. Where small quantities of purified water are required for cell culture, sterile Water For Injection[5] (WFI) or other medically approved pure-water preparations may be used (Stacey and Davis, 2007[8]).

Heating, cooling and humidity should be adequate for the comfort of laboratory occupants and for operation of laboratory equipment, and should not adversely affect test system survival/behaviour and test item stability. For example, in some cases (e.g., preparation of microscopic slides) specific humidity might be required. Desiccation of cell culture media should be avoided and most modern incubators will have humidification systems installed as standard.

Many tissue culture media components are sensitive to white light (especially sun light), with blue wavelengths being of particular concern. Filters can be used in the room, on the windows and laminar flow cabinet light to reduce this exposure where necessary.

Mid to long term storage of media is usually best at temperatures below ambient laboratory temperatures. Accordingly, an optimal solution may be to store all cell culture media at 2°C to 8°C (refrigerator) or frozen (freezer) or as recommended by the manufacturer. There may be exceptions to this general rule but the manufacturers' instructions should always be consulted.

3.5. Quarantine for new test systems

New cells and tissues should be quarantined in the laboratory or in storage until determined to be free of contaminating microorganisms (Figure 3.1). However, exceptions may be made for specific cases: e.g., human blood samples cultured for chromosomal aberration test cannot be stored and must be used on the second day after receipt. It is important for those cases where quarantine is not possible to have supplier documentation, e.g., CoA indicating freedom of contamination. There may also be some cases where the CoA or proof of freedom of contamination is not provided directly with the test system, e.g., some 3D tissue. In these cases the contamination aspects should be assessed in parallel with the work and all work performed in a controlled environment. The test facility should not release any data acquired with this test system until freedom of contamination has been proven. Regular tests to identify contamination of microorganisms during the subsequent cell and tissue culture life cycle, including cell banking, are recommended (Section 5.7).

Early checks of cell authentication (Section 5.6) are also recommended to avoid wasted time and resources on unauthentic cell lines. If separate laboratories/hoods/incubators are not available, steps should be taken to minimise the risk of spreading contamination (Geraghty et al., 2014[4]). Alternatively, other steps can be taken to minimise contamination risks, such as handling the quarantine cells last on each day, rigorous post-manipulation disinfection of the work areas and placing cultures for incubation in a filter-sealed container into the general incubator (Geraghty et al., 2014[4]) (Any area used for the handling of quarantined materials should be routinely cleaned after each use, using a suitable disinfectant. Cells procured from a cell bank may be accompanied with a certificate of analysis which may list the contamination checks performed and provide details of testing methods. At a minimum, a mycoplasma test (Section 5.9, table 9) should be performed upon receipt and cell cultures carefully observed for evidence of contamination.

Notes

1. Refers to prepared samples (e.g., cells treated with test, reference or control items)
2. Separate rooms or containment areas (such as PCR workstation, laminar flow cabinet).
3. See: https://www.gov.uk/government/publications/smi-q-4-good-laboratory-practice-when-performing-molecular-amplification-assays
4. See: https://www.gov.uk/government/publications/smi-q-4-good-laboratory-practice-when-performing-molecular-amplification-assays
5. Also known as Water for Irrigation (WFI)

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