Quality Control of Cellular Therapy Products and Viral Vectors

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1 Introduction

Quality control is defined as "a system for verifying and maintaining a desired level of quality in an existing product or service by careful planning, use of proper equipment, continued inspection, and corrective action as required" [1]. In contrast, quality assurance has a broader definition as "a system for ensuring a desired level of quality in the development, production, or delivery of products and services" [1]. Regulatory authorities place a great deal of importance on product quality, and in its Guidance on the Quality Systems Approach to Pharmaceutical CGMP Regulations [2], the FDA has stated that "Every pharmaceutical product has established identity, strength, purity, and other quality characteristics designed to ensure the required levels of safety and effectiveness. For the purposes of this guidance document, the phrase achieving quality means achieving these characteristics for a product."

The testing required for cellular therapy and gene therapy products has been described in two guidance documents for FDA reviewers and sponsors. The first "Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)" [3] provides a guide to FDA reviewers on what to expect in the Chemistry, Manufacturing and Control (CMC) section of an Investigational New Drug (IND) application to perform a clinical trial using a cellular therapy product. The second "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) [4] provides similar information for gene therapy products. These guidances form the basis of this chapter.

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2 Quality Control (QC) of Somatic Cell Therapy Products

The somatic cell therapy CMC guidance [3] contains sections on product manufacturing and product testing. The testing section recommends that this should include, but not be limited to, microbiological testing and assessments of other product characteristics, such as identity, purity, and potency.

2.1 Microbiological Testing

2.1.1 Sterility

It is recommended that microbiological testing should be performed on cell banks, in-process intermediates, and the final product(s). The FDA indicates that the 21 Code of Federal Regulations (CFR) 610.12 method should be used [5]. This method is described in the United States Pharmacopoeia (USP) <71> [6]. If an alternative method is employed, its suitability should be determined and must be equal or greater than the assurance provided by the recommended method. The two most popular rapid methods are the Bactec system from Becton Dickinson [7] and the BacT/ALERT system from bioMérieux [8]. In 2008, the FDA initially published a guidance on the validation of rapid sterility testing methods, but this was withdrawn in 2015 [9]. Our facility has performed an internal validation of the use of the Becton Dickinson Bactec rapid sterility testing method, but this has not been submitted to the FDA (the Agency). In spite of this, the agency has allowed us to use this method for sterility testing of cell therapy products. We do, however, include a modification of the CFR method in our sterility testing. We incubate the aerobic and anaerobic Bactec cultures for 14 days and the fungal cultures for 28 days. We do have some INDs where the FDA has allowed us to release the products after 7 days of incubation. Comparisons of the various methods have been published [10, 11]. The FDA is now encouraging the use of rapid test systems [4].

If antibiotics are used during manufacturing, there should be documentation that these were removed before sterility was tested. If they cannot be removed, then a bacteriostasis/fungistasis assay should be performed as described in USP <71> [6].

Sterility tests should also be performed at critical points during manufacturing. For cryopreserved products which are not manipulated before administration, the testing should be performed prior to cryopreservation. If the products are thawed and manipulated, e.g., washed, the sterility testing should be repeated. These should include a Gram stain to provide immediate results before administration, and a 14-day culture, with provision to report the results to the recipient's physician as soon as they are obtained, and a plan to deal with positive results from the 14-day culture. The plan should also address the results of an investigation into the positive result, corrective actions. An information amendment should be submitted to the IND within 30 calendar days after receipt of the initial positive result [3]. If the

recipient experiences any serious or unexpected adverse reactions to product administration, the FDA should be notified not more than 15 days after receipt of the information in an IND safety report [3].

In other cases where the product is administered before the results of the culture test are available, it is recommended that a sample be sent for sterility testing 48–72 hours before the final harvest or after the last feeding of the cultured cells. This should be combined with a Gram stain on the final formulated products and the routine 14-day culture test [3].

2.1.2 Mycoplasma

The FDA identified the sources of mycoplasma infections as any animal serum used during manufacturing and the culture environment, particularly if open systems are used [3]. They recommend testing at a manufacturing stage when contamination is most likely to be detected, e.g., after pooling cultures but before cell washing.

The test should be performed on both the cells and the supernatant. Where there is insufficient time to perform the culture-based test [12], they suggest the use of a PCR-based or rapid detection assay. There are several approved PCR assays. These include MycoSEQ™ [13] from Thermo Fisher and MycoTOOL™ from Roche [14]. If a non-approved method is used, a validation should be performed to show that the method is comparable to the culture technique in terms of both sensitivity and specificity. An alternative to the PCR assays is the MycoAlert™ assay system from Lonza [15]. This is a biochemical test that exploits the activity of mycoplasmal enzymes which are found in all six main mycoplasma cell culture contaminants.

Viable mycoplasma in the test sample is lyzed, and the enzymes react with a test substrate that catalyzes the conversion of ADP to ATP. This is then transferred to a light signal via the luciferase enzyme, which is detected using a luminometer. By measuring the level of ATP in the sample before and after the addition of the substrate, a ratio is obtained that indicates the presence or absence of mycoplasma. Since this test has not received full regulatory approval, a validation against the culture method is recommended. Recently, bioMérieux announced the launch of their BIOFIRE®MYCOPLASMA assay [16].

In cases where there may be interference with the mycoplasma assay by constituents of the final product, a mycoplasmastasis assay should also be performed [17].

In 2019, the FDA issued a proposed rule to remove the testing method for mycoplasma detection in virus harvest pools and control fluid pools of live and inactivated virus vaccines produced from in vitro living cell cultures, as currently required by 21 CFR 610.30 [18]. This indicates a possible flexibility by the agency in considering other test methods.

2.2 Adventitious Agent Testing

The FDA advises consultation of their "Points to Consider in the Characterization of Cell Lines used to Produce Biologicals" [19] and the International Conference on Harmonization (ICH) Guidance Q5A "Guidance of Viral Safety Evaluation of Biotechnology Products derived from cell Lines of Human and Animal Origin" in determining what testing may be required for other adventitious agents [20]. It is generally true to say that viral testing is not usually required for cell therapy products derived from autologous or allogeneic lines derived from donors who have undergone donor eligibility testing [21], unless these are used to derive continuous cell lines, e.g., mesenchymal stromal cells. If a cell line is produced, it is sometimes possible, if the line is used for a very small clinical trial, to perform only in vitro adventitious virus testing. More commonly, however, the line must be tested for a panel of specific viruses by PCR, in addition to in vitro and in vivo adventitious viral testing [3].

3 Identity Testing

Identity testing is performed to verify that the product is the correct one and to distinguish it from other products manufactured in the facility. This may be accomplished using a variety of tests. The most commonly used is immunophenotyping by flow cytometry. In most cases, the immunophenotype of the final product should meet specifications for the expression, or lack of expression, of particular CD markers. During early-phase clinical trials, the expression levels may be relatively generous, e.g., <2% CD19, >70% CD3, but as the trials progress, tighter expression levels will be expected. It is normal to incorporate a number of both positive and negative markers for the analysis, and these should be reviewed by the regulatory agency. Immunophenotyping is also used to determine the relative cell purity in the final product. These assays should be performed by an accredited flow cytometry laboratory.

Another assay that may be used to establish product identity is typing for genetic polymorphisms, such as blood type, which may be included [3], although this is not highly specific. Instead HLA identity between the cell donor and the final product is preferable for cells expressing HLA antigens.

4 Purity

Purity is defined as relative freedom from extraneous material in the final products, whether or not that material is harmful to the intended recipient or deleterious to the product [22]. Impurities consist of endotoxin, residual proteins, or peptides used to

pulse or stimulate cells, reagents/components used during manufacturing, e.g., cytokines, antibodies and serum, and unintended cellular phenotypes.

4.1 Residual Contaminants

The final product should be tested for residual proteins and peptides used during manufacturing and reagents used during culturing and purification, e.g., cytokines, growth factors, antibodies, beads, and serum. The final product should also be tested for cell debris and other immunophenotypes. The assays to be used and the specifications for product release must be described in the IND [3].

4.2 Endotoxin

The traditional method for testing for pyrogenicity was the rabbit pyrogen test [23]. This, however, has been largely replaced by the limulus amebocyte lysate test. This in turn has been automated into a rapid assay using an FDA-approved device – the Endosafe® nexgen-PTS™ system with FDA-licensed Endosafe® LAL cartridges [24]. The specification normally approved is <5.0 EU/kg body weight/hr. for the administered product. For intrathecally administered product, the limit is 0.2 EU/kg body weight/hour [3]. The use of the Endosafe system provides a rapid turnaround time that is suitable for all products.

5 Potency

Potency assays should be performed on the product at all stages of clinical trials; however, by the start of Phase 3, the assay should consist of in vivo or in vitro tests that measure appropriate biological activity. This assay must be validated prior to product licensure. If it is not possible to develop a quantitative biological assay, then a quantitative physical assay can be used, if it is performed in conjunction with a qualitative biological assay and correlates with it [3].

For early-phase assays, where the mechanism of action of the product in vivo may not be clear, it is normal to use an assay which quantitates possible effector mechanisms, e.g., cytokine release, cytotoxicity assays, etc. These should be discussed with the regulatory agency at the time of protocol submission.

6 Other Assays

6.1 General Safety Assay

Cellular therapy products are not required by the FDA to undergo general safety testing [3].

6.2 Viability

There should be a specified release criterion for cell viability. The FDA usually requires a benchmark of 70% [3]. If this cannot be achieved, then it should be demonstrated that the dead cells do not adversely affect safe administration of the product or its therapeutic effect.

A number of assays can be used to determine cell viability. Commonly used are the dye exclusion tests, e.g., trypan blue exclusion [25]; however, this is subject to variability by the observer and does not always detect sublethal damage that may occur subsequently. Assays using flow cytometry, e.g., staining with 7 aminoactinomycin D (7-AAD), provide a more reproducible assessment of viability [26]. Apoptosis can also be measured by staining for annexin [27]. Devices are now available for performing automated cell counts and viability measurements, e.g., the Cellometer Auto 1000 Bright Field Cell Counter from Nexcelom [28] and the NucleoCounter® NC-202™ from Chemometec [29]. These should be validated before use.

The FDA usually bases the viability release criteria on the viability at cryopreservation. It should be confirmed that this criterion can be met after thawing by validating the method used for freezing.

6.3 Cell Dose

The minimum number of viable and functional cells to be administered should be specified. It is recommended that the FDA be informed of the maximum dose that has been established based on preclinical experiments.

7 Product Stability

The stability of the product must be evaluated during the early phases of the clinical trial to determine that it will be stable over the entire trial period. The ICH has published guidelines to help with these studies: “ICH Guideline Q1E ‘Stability Testing

of Biotechnological/Biological Products' [30], and Guideline Q1A(R2) 'Stability Testing of New Drugs and Products'" [31].

The FDA requires stability testing at all phases of the IND. The test protocol should include measures of sterility, identity, purity, quality, and potency [3]. The test methods must be described, together with the sampling time points (including a time zero point), the test temperature, and any other appropriate information. The sterility should be tested at zero time, the end of the stability study, and at one intermediate time point [3].

7.1 In-Process Stability Testing

The stability of frozen products should be regularly assessed.

7.2 Final Product Stability Testing

The stability of the final product between the time of product formulation and administration should also be established. This should be done at the appropriate temperatures and at time points consistent with the anticipated holding times. It should include stability during shipment of the product to other sites, preferable under stressed conditions [3].

8 Quality Control for Manufacture of Viral Vectors

Due to the complexity of manufacturing viral vectors, the quality control testing required is more extensive. The FDA requirements are outlined in the guidance "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications" [4]. This guidance clearly outlines the requirements for manufacturing and testing manufacturing intermediates, e.g., master and working cell banks as well as the final drug product. The following sections describe the testing to be performed:

8.1 Master Cell Banks (MCB)

Qualification of an MCB should include testing for sterility, mycoplasma, and adventitious viral agents, in addition to testing for retroviral contamination using reverse transcriptase assays and transmission electron microscopy. The MCB should be tested vigorously for adventitious viral agents (Table 1 for retroviral and

Table 1 Quality control testing performed on cellular therapy products

Testing parameter	Test performed
Microbiological testing	*Sterility* 21 CFR part 610.12 USP <71> ICH Q4B annex 8 sterility test Bactec rapid test (Becton Dickinson) BacTALERT rapid test (BioMerieux) Gram stain *Mycoplasma* Culture methods 21 CFR 510.30; USP <63> WHO nucleic acid test PCR tests MycoTOOL (Roche) MycoSEQ (Thermo Fisher) Other. MycoAlert (Lonza)
Purity	*Residual contaminants* Specific assay, e.g., for residual antibiotics, cytokines, etc. *Pyrogenicity/endotoxin* 21 CFR 610.13(b): rabbit pyrogen test USP <85>: limulus amebocyte lysate assay, ICH guideline Q4B: Limulus amebocyte lysate assay Endosafe: automated limulus amebocyte lysate assay
Identity	Immunophenotype. Genetic polymorphisms.
Potency	In vivo and/or in vitro assay for functionality. Required to correlate with biological activity by Phase 3 trial.
Viability	Dye exclusion assay Stain + Flow cytometry Automated systems
Dose	Manual cell count Automated cell count
General safety	Not required
Stability	Test for sterility, identity, purity, quality, and potency over storage period and after preparation for administration

adenoviral vectors). This may necessitate the use of viral clearance studies to remove and inactivate adventitious agents. Cell lines that have been exposed to bovine (serum) and porcine (trypsin) components should be tested fort the relevant adventitious viruses [4].

8.2 Working Cell Banks (WCB)

The WCB is derived from the MCB, and its testing is less extensive. Generally testing for sterility, mycoplasma, identity, and in vitro adventitious virus is sufficient (Table 2 for retroviral and adenoviral vectors) [4].

8.3 Master Viral Banks (MVB)

MVB should be tested for the absence of contamination, e.g., sterility, mycoplasma, and in vivo and in vitro adventitious viral agents. It should be tested for the presence of replication-competent virus in replication-incompetent vectors. The viral titer or concentration should be determined and the presence of transgene activity. The identity of the vector and the therapeutic transgene should be tested, and the correct genetic sequence confirmed (Table 2 for retroviral and adenoviral vectors) [4].

8.4 Working Viral Banks (WVB)

The WVB is derived from the MVB, and testing is generally limited to sterility, mycoplasma, identity, and in vitro adventitious agents (Table 2 for retroviral and adenoviral vectors) [4].

8.5 Final Vector Product

8.5.1 Process-Related Impurities

The final vector (drug substance) should be tested for manufacturing impurities, e.g., residual cesium chloride for adenoviral vectors, host cell DNA, cytokines, growth factors, etc. The manufacturing process should be designed to reduce non-vector DNA contamination. It is difficult to find an FDA specification for residual DNA, but the World Health Organization recommends a limit of 10 ng/dose [32]. The size of the DNA should preferably be below that of a functional gene to minimize its biological activity. Host cell protein must also be assayed [33].

Table 2 Suggested testing during manufacture of retroviral and adenoviral vectors

Master cell bank	Working cell bank derived from tested master cell bank	Master viral banks	Working viral bank derived from tested master viral bank
Sterility	Sterility	Sterility	Sterility
Mycoplasma	Mycoplasma	Mycoplasma	Mycoplasma
Endotoxin	Endotoxin	Endotoxin	Endotoxin
Identity	Identity	In vivo adventitious virus	Identity
Cytomegalovirus	In vitro adventitious virus	In vitro adventitious virus	In vitro adventitious virus
HIV-1 and 2		Replication-competent virus	Replication-competent virus
HTLV-1 and 2		Viral titer or concentration	Viral titer or concentration
Human herpes virus 6, 7, and 8		Transgene activity	Transgene activity
JC virus		Identification of viral vector and therapeutic transgene, e.g., by southern blot or restriction endonuclease	Identification of viral vector and therapeutic transgene, e.g., by southern blot or restriction endonuclease
BK virus		Correct genetic sequence, e.g., by full sequencing (for 40 kb or smaller), annotated sequence analysis For >40 kb, sequence analysis including testing by restriction endonuclease analysis. Sequence analysis of gene insert, flanking regions, and any regions modified or deleted and susceptible to recombination For integrating vectors, DNA sequencing on integrated vector	
Epstein-Barr virus			
Human parvovirus B19			
Human papilloma virus			
Hepatitis C			
Bovine/porcine virus if appropriate	Bovine/porcine virus if appropriate		
Identity by genetic analysis (e.g., STR)			

(continued)

Table 2 (continued)

Master cell bank	Working cell bank derived from tested master cell bank	Master viral banks	Working viral bank derived from tested master viral bank
Reverse transcriptase			
Transmission electron microscopy			
Additional tests Stability over time Tumorigenicity	Stability over time	Stability over time	Stability over time

8.5.2 Product-Related Impurities

Product-related impurities consist of noninfectious particles, empty capsid particles and replication-competent virus contaminants, etc. For genetically modified cells, impurities would include unmodified target cells and nontarget cells. Where possible, their presence should be enumerated.

8.5.3 Testing of Vector Product [4]

Testing on the final vector product should include microbiological testing, such as bioburden or sterility testing as appropriate, mycoplasma and adventitious viral agent testing. Rapid tests for mycoplasma and sterility should be qualified/validated to ensure their suitability. Replication competence should be tested for nonreplicating vectors at various points during the manufacturing procedure. The assay results should be supported by data demonstrating the accuracy, reproducibility, sensitivity, and specificity of the test method, and the assay should include the appropriate controls.

8.5.4 Testing of Genetically Modified Cells

In general, the testing of genetically modified cells closely follows that of nonmodified cells; however, some additional testing is normally required. This includes the vector copy number in the transduced cell population. A general specification of <5 copies per cell is normally acceptable. There should also be some test for evidence of satisfactory modification. These may include expression of the transgene detected by flow cytometry and/or evidence of functionality of the gene in a potency assay, e.g., confirmation of production of a cytokine in response to specific stimulation. The acceptability of the proposed test should be cleared with the regulatory authority.

9 Stability Testing on Vector Intermediates and Final Products

Stability testing should be performed on all of the various intermediates involved in the manufacture of a genetically modified therapeutic cell product. These include the master and working cell banks, the master and working viral banks, the final vector product, and the genetically modified cells. These will include stability of the cells or vector during long-term storage and between thawing and administration of the vector or gene-modified cells. The test protocol should include measures of sterility, identity, purity, quality, and potency [3, 4].

10 Release of Products for Administration

Products that are prepared using more-than-minimal manipulation (e.g., cultured, genetically modified, activated, etc.) are released under a Certificate of Analysis (CofA) [2, 34], the contents of which have been approved by the regulatory authority that has cleared the clinical trial. The CofA contains the appropriate testing specifications for microbiological testing, purity, identity, potency, and viability, together with information of the tests used (preferably including their specificity and sensitivity), and the results obtained. This information is assembled by the quality control laboratory and transferred to the quality unit, which reviews it and generates the CofA, which is signed by the quality director (or designee) and a Laboratory Medical Director. At this stage, the product may be made available for distribution and administration.

Products that are minimally manipulated still undergo basic quality testing, e.g., sterility, endotoxin, identity, viability, etc., but are not usually released under a CofA.

11 Other Quality Control (QC) Responsibilities

In addition to product testing, the QC laboratory usually has other responsibilities [35]. These may include performing environmental monitoring of the facility, managing cleaning schedules and disinfectant rotation, selecting external testing vendors, shipment of samples, and collation of results. The QC unit must consistently evaluate new testing technologies and introduce them where appropriate.

12 Conclusions

The quality control unit plays an essential role in the release of therapeutic products by performing in-process and final product testing. Information that they provide can also help identify improvements in manufacturing procedures and identify weak points during operations. As such, they play a vital role in GMP operations.

This chapter represents a point in time, and investigators are always encouraged to contact the appropriate regulatory authority to determine the current regulations for the release of cellular therapy and viral vectors for clinical use.

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