Safety Testing for C&G Therapy Products

Available technologies for rapid testing offer possibilities for quick delivery of the genetically modified product for diseases that have long been considered incurable

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The growth of advanced therapy medicinal products (ATMPs), such as gene and cell therapy products, has made significant progress in the treatment of cancer, rare diseases, and autoimmune disorders (1-3). With the promise to become a very real solution for these previously untreatable diseases, different strategies for gene and cell therapies are highly developed, and biopharma companies are in a race to clinical application while some products have already reached market authorisation. Technologies for editing genes and correcting inherited mutations, the recruitment of stem cells to regenerate tissues, and the stimulation of powerful immune responses to fight cancer are also contributing to the investment in these therapies.

Which are the Manufacturing and Quality Needs?

Manufacturing and product testing are key elements for the success of a reliable and efficient strategy of product development for cell and gene therapies. There are opportunities to improve both manufacturing efficiency and characterisation/final testing strategies by moving to automation and using new technologies for analytical methods development. Shortening the release timelines that usually exceed the production process duration will reduce production costs and enhance the access to patients and product reimbursement.

Orphan Drugs and Oncologic Therapies

Orphan drugs for rare disease have been the pioneer products in the field. Made by using patients' own cells, which are genetically engineered and reinserted into the patient, these 'autologous' products require complex manufacturing processes and release strategies. Small batch manufacturing and a limited amount of sample quantity, dedicated to testing, are the main limitations. Besides, complex release panels lead to very challenging plans for the timely arrival of the therapies to the patients.

CAR T cell therapies, for cancer treatment, have the major advantage that, for some applications, they can be made using allogeneic cells. This decreases the cost of goods and time concerning cell therapies. Large batch manufacturing, easily available with allogeneic cells, can provide an appropriate quantity of samples for testing and facilitate the quality control strategy.

Limiting Factors During ATMP Release Phase: Which Technologies Can Play a Crucial Role?

The shortening of release timelines continues to play an important role for these therapies with both autologous and allogeneic cells. Standard product safety testing is the limiting factor during the release phase: sterility, mycoplasma, and viral contaminants are critical attributes that require the specific employment of operator skills and cell-based analytical methods with long turnaround times. The solution to proactively reduce release timelines and fulfil regulatory expectations will be the use of the rapid methods for sterility, mycoplasma, and viral contaminant detection, together with the complete analytical methods validation, required since the beginning of the investigational medicinal product clinical trials (4).

Methods for Sterility Testing: Compendial vs Rapid Methods

During manufacturing of ATMP cell-based products, the risk of potential contamination is reduced by testing this critical quality attribute at different steps of the production process. Both compendial and rapid sterility tests can be used and require either validation or a product specific suitability study. There are several differences regarding principles, temperatures, incubation time, volume of substance to be inoculated, or number of items to be tested (see **Table 1,** page 24).

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Method	References	Principle	Temperature	Incubation	Minimum quantity
Compendial sterility	EP 2.6.1 USP <71>	Visual examination of media	20-25°C for aerobic microorganism 30-35°C for anaerobic microorganism	14 days	-10% for volumes higher than 100ml (but not less than 20) -Half volume for samples between 1-40ml (no less than 1ml)
Rapid sterility (BacT/ ALERT 3D)	EP 2.6.27	Colorimetric detection of CO_2 presence	Different temperature settings possible	7 days	-1% for volumes higher than 100ml -100µL for samples between 1-10ml
Rapid sterility (BACTEC)	EP 2.6.27	Fluorescence detection of CO_2 presence	Different temperature settings possible	7 days	-1% for volumes higher than 100ml -100µL for samples between 1-10ml

Table 1: Comparison between compendial and rapid sterility methods

Compendial tests consist of either membrane filtration or direct inoculation of the sample into two different media, for growth of aerobic and anaerobic microorganisms, followed by incubation at different temperatures for 14 days. During incubation, visual examination of media is performed by checking turbidity. For liquid samples as for ATMPs, the volume for testing varies depending on quantity per container as summarised in **Table 1**.

Rapid methods such as BacT/ALERT 3D or BACTEC consist of direct inoculation of the sample into aerobic and anaerobic media, followed by incubation in an instrument, for at least seven days, at different temperatures, depending on different approach sets. Carbon dioxide produced by microbial growth is detected with a colorimetric or fluorescent method for BacT/ ALERT 3D or BACTEC respectively. These systems are not dependent on operator skills, replacing the visual check with automated, instrument-based analysis. The total inoculum volume is reduced compared to compendial test (see **Table 1**).

Overall, both methods are accepted by regulatory authorities for ATMPs. Viral vector-based products can be subjected to an effective process of filtration and the compendial sterility test can be applied. Rapid methods are more suitable for cell-based products considering the advantage to shortening the release timelines for patients with no alternative therapeutic solutions.

Rapid Mycoplasma Methods

Mycoplasma species are bacteria that can infect cell cultures and thereby pose a potential threat to patients receiving cell or gene therapy products. Testing is required at different stages of the production process.

The conventional mycoplasma tests, according to EP 2.6.7 and USP <63>, are highly sensitive methods but require a minimum incubation period of 28 days, which is problematic in the release of ATMPs.

Currently several nucleic acid amplification technique (NAT) based methods are available to reduce the turnaround time drastically (selection in **Table 2**). Such NAT-based methods must be validated according to EP 2.6.7, 2.6.21, and USP <1071> to prove equality to the conventional mycoplasma test methods in order to replace them (5-6). To serve as an alternative for the culture method and the indicator cell culture method, the NAT test system must prove sensitivity of 10 CFU/ mL and 100 CFU/mL respectively. A possible challenge here may be to translate the detection limit in CFU/ mL to an equivalent limit in genome copies/mL for each mycoplasma species tested.

In direct quantitative polymerase chain reaction (qPCR) methods, the DNA isolation may be challenging. Especially in vaccines or plasmid-based products, the drug product itself may cause interference during DNA isolation or qPCR. Therefore, system suitability should always be performed before the method is used for release testing. Alternative NAT methods that could circumvent DNA isolation, such as digital droplet PCR might serve as a suitable alternative for DNA-rich drug products.

Molecular Detection Techniques for Viral Contaminants

Viral contamination is a risk common to all cell line derived biotechnology products (7). Adventitious viral contaminations can be introduced through starting materials, such as cell line or media, or during production.

Kit/test	Technology	Method	Start to results	Hand-on time
Venor®GeM (Minerva Biolabs)	Direct PCR	DNA isolation – PCR – agarose gel	8-10 hours	4-6 hours
MycoSeq™ (Thermo Fisher)	Direct qPCR using SYBR green	DNA isolation (manual of automated) – multiplex qPCR using SYBRgreen (limit test)	8 hours	4-6 hours
Venor®GeM (Minerva Biolabs)	Direct qPCR using Taqman probes	DNA isolation – multiplex qPCR using primer/probe sets (limit test)	6-8 hours	3-4 hours
Mollicute screening	Cell-culture enrichment followed by NAT	Culture in broths of conventional test -> DNA isolation multiplex qPCR using SYBRgreen (limit test)	5-8 days	6-8 hours
CytoInspect™ (Grenier-Bio)	DNA microarray	DNA isolation -> Touchdown PCR created labelled fragments -> DNA amplicon hybridisation	6-8 hours	4-6 hours

Table 2: Comparison of available NAT based methods for mycoplasma testing

The testing strategy applied to traditional biopharmaceuticals includes testing of starting materials and production intermediates followed by inactivation and removal of contaminating viruses. However, the steps to inactivate and remove viruses may not be suited when the drug products themselves are viruses or living cells.

An appropriate testing strategy for novel starting material becomes vital for these kinds of ATMPs.

The ICH Q2(R1) guideline describes the parameters that must be validated for analytical methods (8). The parameters vary depending on the type of test to be

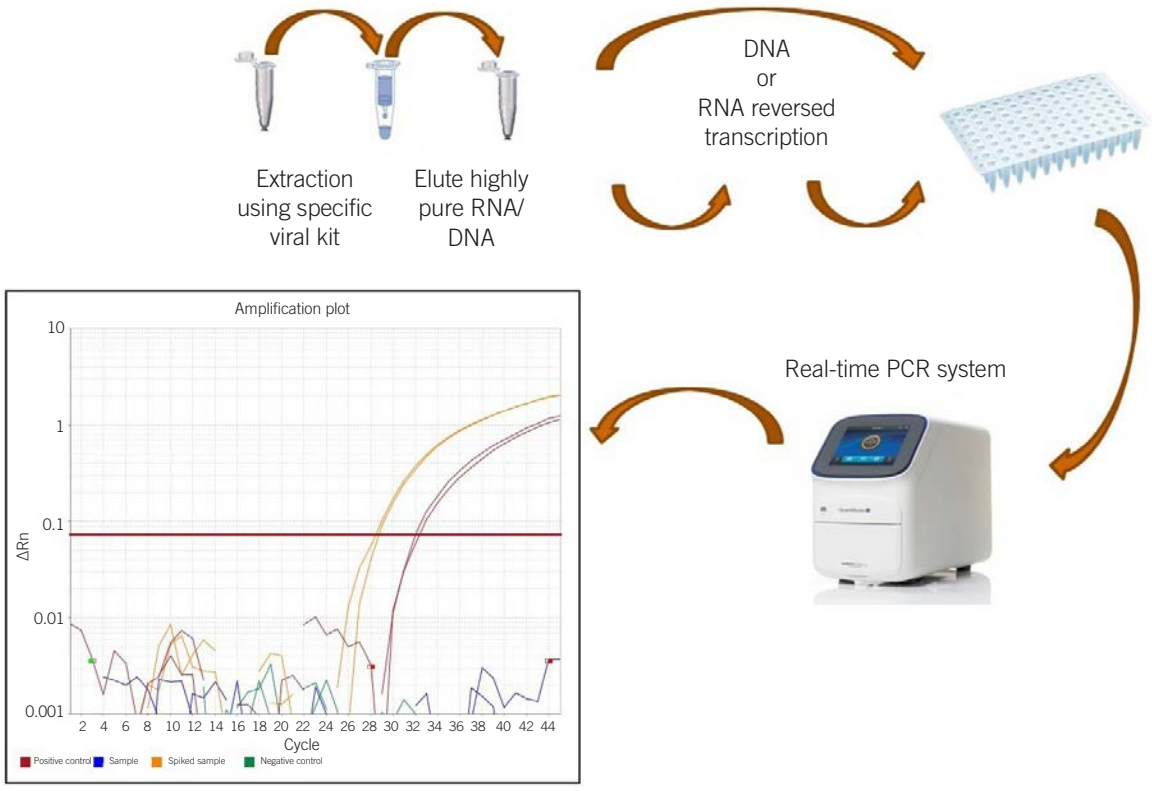

Figure 1: Overview of the analytical steps for viral detection by qPCR. In the amplification plot of a routine test, only signal for positive control (red) and spiked-sample (yellow) are detected. No amplification should be detected in sample (blue) and negative control (green)

Parameter	Determination by test requirement	Test type	
		Quantitative	Limit
Accuracy	Comparison of observed and expected result	+	-
Specificity	Primer/probe show no homology with non-related sequences. No amplification signal for virus not related to the target virus	+	+
Precision:			
- Repeatability	Precision under sample operating conditions (intra-assay precision)	+	-
- Intermediate precision	Variation within one laboratory (different days, operators, equipment)	+	-
- Reproducibility	Variation between laboratories	+ (only in tech transfer)	-
Limit of detection	Minimum amount of viral standard detected in at least 95% of inoculated samples	+	+
Limit of quantitation	Lowest amount of viral standard that can quantitatively be determined	+	-
Robustness	No difference expected in results obtained after variations have been applied to the method.	+	-
Linearity	The ability of the test to obtain results which are directly proportional to the concentration (amount) of virus in the sample	+	-
Range	Interval between lower and upper limit for which a suitable level of precision, accuracy and linearity is established	+	-

Table 3: Validation parameters for detection of viral DNA/RNA by real time PCR

validated (see **Table 3**). Viral testing methods are frequently validated using dummy samples like matrix spiked with either the product or reference material.

Typical cell culture-based techniques, such as *in vitro* adventitious virus assay (AVA), continue to be used to assess quality and safety. Those techniques have undergone some radical changes to reduce their timing and improve their sensitivity. However, they are slowly being replaced by molecular detection techniques such as qPCR. They are sensitive, selective, and fast, making them the preferred method for testing all materials.

qPCR is a well-established and powerful tool for the rapid and accurate detection of viral DNA or RNA (5). Briefly, the steps in virus qPCR include nucleic acid extraction and, for RNA viruses, reverse transcription, then amplification of a conserved region of the viral genome by specific primers, and detection by fluorogenic probes (see **Figure 1,** page 25). Current qPCR panels include targets for human, porcine, bovine, and simian viruses (9). These panels are fundamental to test final products, intermediates, and raw materials used for cell culture. Also available are the fluorescent product enhanced reverse transcriptase methods for the detection of retroviral contaminations.

Conclusions

Gene and cell therapy products provide new possibilities for replacing a malfunctioning gene within the cells adversely affected by the disease. Depending on the origin of raw and starting material there might be a risk related to transmissible diseases.

When designing the quality strategy for product safety investigation, the following aspects are crucial:

1. Early definition and validation of the appropriate sensitive testing panel
2. Identification of rapid methods for the timely delivery of the product to the patient

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